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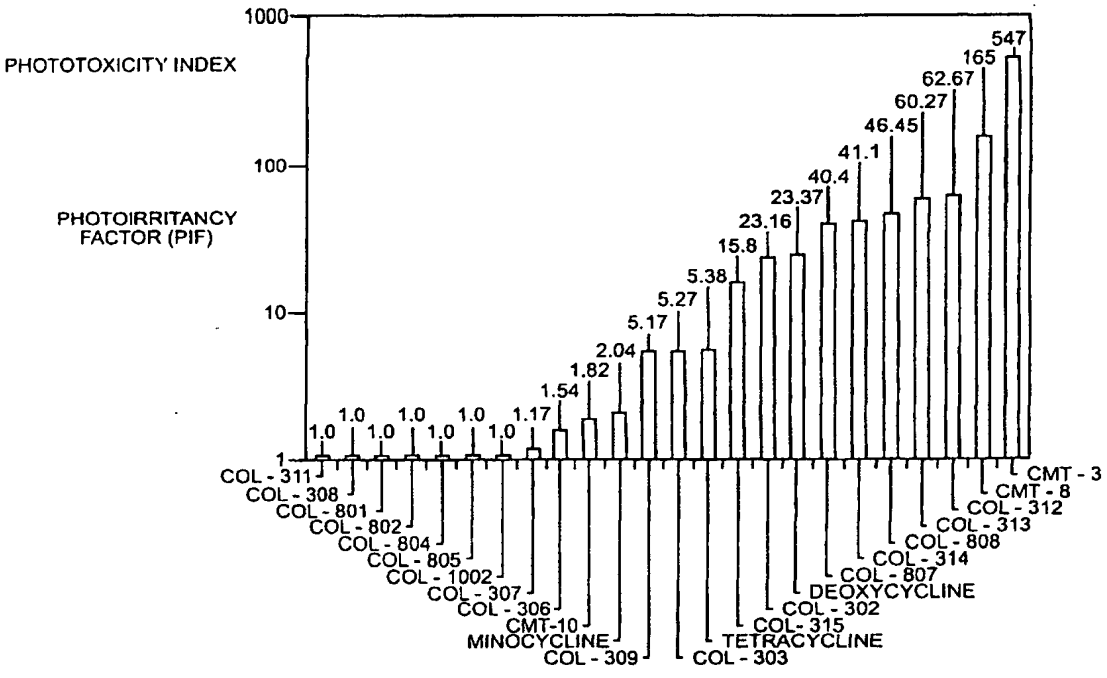

(57) Abstract: A method for treating an allergic reaction other than asthma in a mammal need thereof comprising administering to said mammal a tetracycline compound in an amount that is effective to treat said allergic reaction.

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METHODS OF TREATING ALLERGIC REACTIONS

The present application claims benefit of U.S. provisional application serial no. 60/382,127, filed May 20, 2002, which is incorporated herein by reference.

BACKGROUND OF THE INVENTION

An allergic reaction is a condition in which the immune system reacts with hypersensitivity to a substance or substances, called allergens. Allergic reactions develop through a process called sensitization. Sensitization can occur on first contact, or over a brief period, or even through repeated exposure over several years. Allergens are generally quite harmless to individuals who do not have the particular allergy.

Every time an allergic person is exposed to a particular allergen it is likely that the same response will result, and the reaction may become more severe over time. The level of exposure to the substance at which an allergic reaction will be triggered is the person's allergic threshold.

Allergic reactions can take many forms, from mild to severe. The mild form may involve minor discomfort, such as a rash or indigestion. The severe form may involve extreme irritation of skin or mucous membranes, respiratory distress or anaphylactic shock. In rare cases, an allergic reaction may be fatal.

During the last ten to fifteen years, the prevalence of allergies has dramatically increased in western countries. It has been estimated that at least 15-20% of the population of developed western countries suffer from allergic reactions, such as seasonal rhinitis, urticaria or asthma.

It is believed that upon first exposure to an allergen, plasma cells are stimulated to produce antibodies, e.g., IgE. These antibodies attach to high affinity IgE-receptors (FcϵRI) of mast cells. Mast cells are bone marrow-derived cells of the immune system which reside abundantly in connective tissues and mucosal membranes of the

nose, bronchi, lungs and gastrointestinal tract. The cytoplasm of mast cells is filled with intracellular granules which contain active mediators of inflammation, such as histamine, cytokines; and mast cell proteases, i.e. tryptase, chymase and carboxypeptidase A. Upon activation, mast cell are believed to release their mediators.

The most commonly used drugs to alleviate allergic symptoms are antihistamines. Antihistamines block the type 1 histamine receptors, thereby preventing some of the histamine induced reactions. However, the blocking of histamine receptors may increase some allergen-induced symptoms. The action of histamine receptors on the plasma membrane of mast cells functions as a negative feedback mechanism to inhibit the secretion of, for example, cytokines by activated mast cells. Therefore, while some symptoms of an allergic reaction may be alleviated by blocking the effect of histamine, the inflammatory reaction driven by mast cell derived cytokines may not be inhibited, and may even be increased due to the attenuation of the negative feedback mechanism.

Accordingly, there is a need for an improved method for effectively treating allergic reactions.

SUMMARY OF THE INVENTION

In one embodiment, the present invention provides a method for treating allergic reactions, other than asthma, in a mammal in need thereof. The method comprises administering to the mammal a tetracycline compound in an amount that is effective to treat the allergic reaction. Preferably, the tetracycline compound has substantially no antibiotic activity.

In another embodiment, the present invention provides a method for treating asthma in a mammal in need thereof. The method comprises administering to the mammal a tetracycline compound in an amount that is effective to treat the allergic

reaction, without administering a bisphosphonate compound. Preferably, the tetracycline compound has substantially no antibiotic activity.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the photoirritancy factor (PIF) for some tetracycline compounds. For structure K, the compounds indicated are as follows:

COL	R7	R8	R9
308	hydrogen	hydrogen	amino
311	hydrogen	hydrogen	palmitamide
306	hydrogen	hydrogen	dimethylamino

For structures L, M, N or O the compounds indicated are as follows:

COL	R7	R8	R9
801	hydrogen	hydrogen	acetamido
802	hydrogen	hydrogen	dimethylaminoacetamido
804	hydrogen	hydrogen	nitro
805	hydrogen	hydrogen	amino

For structure P, R8 is hydrogen and R9 is nitro (COL-1002).

Figure 2 shows a Sample Dose Response Curve of the Positive Control Chlorpromazine for use in PIF calculations.

Figure 3 shows a Sample Dose Response Curve for use in MPE calculations.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides methods of treating allergic reactions in a mammal.

An allergic reaction, as defined herein, includes any state of hypersensitivity induced by exposure to a particular allergen resulting in immunologic reactions on subsequent exposure that cause at least one symptom of an allergic reaction. An allergen is any antigenic substance that can induce activation of mast cells in a susceptible mammal in an IgE antibody-dependent manner.

For the purposes of this specification, allergens include all known types of allergens. Some examples of allergens include respiratory allergens, such as grass, pollen, mold spores, nettles, poison ivy, dust mites, dandruff, and animal hair; drug allergens; food allergens, such as cows' milk, eggs, peanuts, strawberries, wheat, shellfish and seafood; foreign substances in the blood stream; insect bites, such as bee stings; latex and sunlight.

Allergies, as used herein, include all known types of allergies. Some examples of allergies include atopic allergy; bacterial allergy; bronchial allergy, i.e. asthma; cold allergy, i.e. cold urticaria, angioedema; contact allergy, i.e. contact dermatitis; delayed allergy; drug allergy; food or gastrointestinal allergy; hereditary allergy; immediate allergy; latent allergy; physical allergy, e.g. photosensitivity, cholinergic urticaria; seasonal allergic rhinitis, i.e. hay fever; atopic rhinitis; polyvalent allergy; allergic conjunctivitis; autoimmune disease; and spontaneous allergy.

Symptoms of allergic reactions include, for example, skin rashes, itching, inflammation or swellings; red and swollen eyes; runny nose; severe nasal inflammation; nasal polyps; wheezing; shortness of breath; gastrointestinal distress, i.e. vomiting, diarrhea; irritation of the mucosa; and anaphylactic shock.

Allergic reactions are caused by exposure of a susceptible mammal to an allergen. Exposure may be caused, for example, by touching the allergen, inhaling the allergen, ingesting the allergen, being in the presence of the allergen, etc.

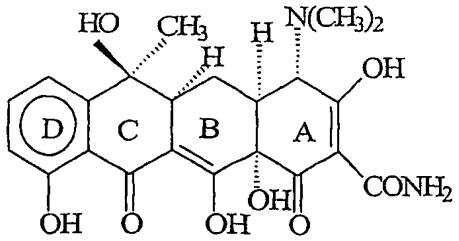
In one embodiment of the invention, a method of treating an allergic reaction, excluding asthma, is provided. The method comprises the administration of a tetracycline compound. The tetracycline compound is administered in an amount which is effective to treat the allergic reaction. Preferably, the tetracycline compound has substantially no antibiotic activity.

In another embodiment of the invention, a method of treating asthma is provided. The method comprises the administration of a tetracycline compound without administering a bisphosphonate. The tetracycline compound is administered in an amount which is effective to treat asthma. Preferably, the tetracycline compound has substantially no antibiotic activity.

Bisphosphonates compounds are related to inorganic pyrophosphonic acid. The bisphosphonates include, as non-limiting examples, alendronate ((4-amino-1-hydroxybutylidene) bisphosphonic acid), clodronate (dichloromethane diphosphonic acid), etidronate ((1-hydroxyethylidene) diphosphanic acid) and pamidronate ((3-amino-1-hydroxypropylidene) bisphosphonic acid); also risedronate ([-hydroxy-2-(3-pyridinyl)ethylidene] bisphosphonic acid), tiludronate, i.e., tiludronic acid ([(4-chlorophenyl) thio]methylene] bisphosphonic acid) and zolendronate.

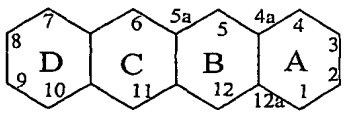
The tetracycline compounds of the present invention can also be used in combination with other antiallergic, anti-inflammatory and anti-asthma drugs.

The tetracyclines are a class of compounds of which tetracycline is the parent compound. The tetracycline compounds include their pharmaceutically acceptable salts. Tetracycline has the following structure:



Structure A

The numbering system of the multiple ring nucleus is as follows:



Structure B

Tetracycline, as well as the 5-OH (oxytetracycline, e.g. Terramycin) and 7-Cl (chlorotetracycline, e.g. Aureomycin) derivatives, exist in nature, and are all well known antibiotic compounds that are suitable for use in the methods of the invention. Semisynthetic antibiotic derivatives such as 7-dimethylaminotetracycline (minocycline) and 6α-deoxy-5-hydroxytetracycline (doxycycline) are also suitable.

Some examples of antibiotic tetracycline compounds include doxycycline, minocycline, tetracycline, oxytetracycline, chlortetracycline, demeclocycline, lymecycline, and sancycline. Doxycycline is preferably administered as its hyclate salt or as a hydrate, preferably monohydrate.

Non-antibiotic tetracycline compounds are structurally related to the antibiotic tetracyclines, but have had their antibiotic activity substantially or completely eliminated by chemical modification, as discussed in more detail below. For example, non-antibiotic tetracycline compounds are incapable of achieving antibiotic activity comparable to that of doxycline unless the concentration of the non-antibiotic tetracycline is at least about ten times, preferably at least about twenty five times, greater than that of doxycycline.

Examples of chemically modified non-antibiotic tetracyclines (CMT's) include, 4-de(dimethylamino)tetracycline (CMT-1), tetracyclinonitrile (CMT-2), 6-demethyl-6-deoxy-4-de(dimethylamino)tetracycline (CMT-3), 7-chloro-4-de(dimethylamino)tetracycline (CMT-4), tetracycline pyrazole (CMT-5), 4-hydroxy-4-de(dimethylamino)tetracycline (CMT-6), 4-de(dimethylamino)-12α-deoxytetracycline (CMT-7), 6-deoxy-5α-hydroxy-4-de(dimethylamino)tetracycline (CMT-8), 4-de(dimethylamino)-12α-deoxyanhydrotetracycline (CMT-9), 4-de(dimethylamino)minocycline (CMT-10). (COL and CMT are used interchangeably throughout this specification.)

Further examples of chemically modified non-antibiotic tetracyclines include Structures C-Z. (See Index of Structures.)

Tetracycline derivatives, for purposes of the invention, may be any tetracycline derivative, including those compounds disclosed generically or specifically in International Application No. PCT/US01/16272, filed on May 18, 2001; and U.S. patent application serial no. 10/274,841, filed on October 18, 2002, which are herein incorporated by reference.

The tetracycline compounds can be in the form of pharmaceutically acceptable salts of the compounds. Pharmaceutically acceptable salts may be prepared from the corresponding tetracycline compounds and an acid or base. The acids may be inorganic or organic acids. Examples of inorganic acids include hydrochloric, hydrobromic, nitric, hydroiodic, sulfuric, and phosphoric acids. Examples of organic acids include carboxylic and sulfonic acids. The organic acids may be aliphatic, aromatic, aliphatic-aromatic or aromatic-aliphatic. Some examples of organic acids include formic, acetic, phenylacetic, propionic, succinic, glycolic, glucuronic, maleic, furoic, glutamic, benzoic, toluic, anthranilic, salicylic, mandelic, embonic (pamoic), methanesulfonic, ethanesulfonic, panthenoic, benzenesulfonic, stearic, sulfanilic, alginic, tartaric, citric, gluconic, gulonic, arylsulfonic, and galacturonic acids. Appropriate organic bases may be selected, for example, from N,N-

dibenzylethylenediamine, chloroprocaine, choline, diethanolamine, ethylenediamine, meglumine (N-methylglucamine), and procaine.

The tetracycline compound is administered in an amount that is effective to treat an allergic reaction, but has substantially no antibiotic activity. A treatment is effective if it causes a reduction or inhibition of the symptoms associated with an allergic reaction.

The minimal effective amount of the tetracycline compound administered to a mammal is the lowest amount capable of providing effective treatment of an allergic reaction. Some examples of minimal amounts include 10%, 20%, 30% and 40% of an antibiotic amount.

The maximal effective amount of the tetracycline compound administered to a mammal is the highest amount that does not significantly prevent the growth of microbes, e.g. bacteria. Some examples of maximal amounts include 50%, 60%, 70% and 80% of an antibiotic amount.

The amount of a tetracycline compound which is administered can be measured by daily dose and by serum level.

Tetracycline compounds that have significant antibiotic activity may, for example, be administered in a dose which is 10-80% of the antibiotic dose. More preferably, the antibiotic tetracycline compound is administered in a dose which is 40-70% of the antibiotic dose.

Antibiotic daily doses are known in art. Some examples of antibiotic doses of members of the tetracycline family include 50, 75, and 100 mg/day of doxycycline; 50, 75, 100, and 200 mg/day of minocycline; 250 mg of tetracycline one, two, three, or four times a day; 1000 mg/day of oxytetracycline; 600 mg/day of demeclocycline; and 600 mg/day of lymecycline.

Examples of the maximum non-antibiotic doses of tetracyclines based on steady-state pharmacokinetics are as follows: 20 mg/twice a day for doxycycline; 38 mg of minocycline one, two, three or four times a day; and 60 mg of tetracycline one, two, three or four times a day.

In a preferred embodiment, doxycycline is administered in a daily amount of from about 30 to about 60 milligrams, but maintains a concentration in human plasma below the threshold for a significant antibiotic effect.

In an especially preferred embodiment, doxycycline hyclate is administered at a 20 milligram dose twice daily. Such a formulation is sold for the treatment of periodontal disease by CollaGenex Pharmaceuticals, Inc. of Newtown, Pennsylvania under the trademark Periostat ®.

The administered amount of a tetracycline compound described by serum levels follows.

An antibiotic tetracycline compound is advantageously administered in an amount that results in a serum tetracycline concentration which is 10-80%, preferably 40-70%, of the minimum antibiotic serum concentration. The minimum antibiotic serum concentration is the lowest concentration known to exert a significant antibiotic effect.

Some examples of the approximate antibiotic serum concentrations of members of the tetracycline family follow. A single dose of two 100 mg minocycline HCl tablets administered to adult humans results in minocycline serum levels ranging from 0.74 to 4.45 μg/ml over a period of an hour. The average level is 2.24 μg/ml.

Two hundred and fifty milligrams of tetracycline HCl administered every six hours over a twenty-four hour period produces a peak plasma concentration of

approximately 3 μg/ml. Five hundred milligrams of tetracycline HCl administered every six hours over a twenty-four hour period produces a serum concentration level of 4 to 5 μg/ml.

In one embodiment, the tetracycline compound can be administered in an amount which results in a serum concentration between about 0.1 and 10.0 μg/ml, more preferably between 0.3 and 5.0 μg/ml. For example, doxycycline is administered in an amount which results in a serum concentration between about 0.1 and 0.8 μg/ml, more preferably between 0.4 and 0.7 μg/ml.

Some examples of the plasma antibiotic threshold levels of tetracyclines based on steady-state pharmacokinetics are as follows: 1.0 μg/ml for doxycycline; 0.8 μg/ml for minocycline; and 0.5 μg/ml for tetracycline.

Non-antibiotic tetracycline compounds can be used in higher amounts than antibiotic tetracyclines, while avoiding the indiscriminate killing of bacteria, and the risk of emergence of resistant bacteria. For example, 6-demethyl-6-deoxy-4-de(dimethylamino)tetracycline (CMT-3) may be administered in doses of about 40 to about 200 mg/day, or in amounts that result in serum levels of about 1.55 μg/ml to about 10 μg/ml.

The actual preferred amounts of tetracycline compounds in a specified case will vary according to the particular compositions formulated, the mode of application, the particular sites of application, and the subject being treated (e.g. age, gender, size, tolerance to drug, etc.)

Preferably, the tetracycline compounds, and the salts thereof, have low phototoxicity, or are administered in an amount that results in a serum level at which the phototoxicity is acceptable. Phototoxicity is a chemically-induced photosensitivity that occurs upon exposure to light, in particular ultraviolet light. Such photosensitivity renders skin susceptible to damage, e.g. sunburn, blisters,

accelerated aging, erythemas and eczematoid lesions. The preferred amount of the tetracycline compound produces no more phototoxicity than is produced by the administration of a 40mg total daily dose of doxycycline.

There are several methods by which to quantify phototoxicity. One method is called photoirritancy factor (PIF). The PIF is the ratio of an IC_{50} value in the absence of light to an IC_{50} value in the presence of light.

In calculating PIF values, the data resulting from the assay procedure can be interpreted by different methods. For example, during the period March 2, 1999 to April 16, 1999, PIF values were obtained using the phototoxicity software and its curve-fitting algorithms available at the time. In the present specification, this earlier phototoxicity calculation is referred to as PIF1. At a PIF1 value of 1, a compound is considered to have no measurable phototoxicity. A PIF1 value greater than 5 is indicative of phototoxic potential of a compound.

As explained in more detail in Example 37 below, 3T3 phototoxicity assay has undergone extensive validation since April 1999, and has now been incorporated into a draft guideline by the Organization of Economic Cooperation and Development (OECD) (Draft Guideline 432). In the present specification, this revised phototoxicity calculation is referred to as PIF2. A PIF2 value of less than 2 is considered non-phototoxic, 2 to less than 5 is considered potentially phototoxic, and 5 or greater is considered clearly phototoxic.

PIF2 values are more refined than the PIF1 values. Qualitatively the differences between the PIF1 and PIF2 values are not significant. For example, the mean PIF1 values for COL 10 and COL 1002 are 1.82 and 1.0, respectively. The mean PIF2 values of COL 10 and COL 1002 are 2.04 and 1.35, respectively.

As explained in the Examples section, PIF values cannot be determined for many compounds. Another method by which to quantify relative phototoxicity is

called mean photo effect (MPE). MPE values can be determined for compounds in virtually all cases. Thus, MPE values are more consistent and reliable than PFE values.

The MPE is a measure of the difference between the cytotoxicity induced by the test chemical in the presence and absence of light. It compares the responses over the range of doses selected using the two dose-response curves produced from the boot-strap analysis of the individual data points (Holzhütter 1995 and 1997). An example is provided in Figure 3 (Peters and Holzhütter (2002)). This method of analysis is particularly suited to cases where the IC_{50} value cannot be calculated for one or both concentration response curves.

MPE values of < 0.1 (including negative values) are considered indicative of a nonphototoxin, values of 0.1 to <0.15 are considered probable phototoxins, and values greater than and equal to 0.15 are considered to be clear phototoxins.

A class of low phototoxicity tetracyline derivatives has less than approximately 75% of the phototoxicity of minocycline, preferably less than approximately 70%, more preferably less than approximately 60%, and most preferably less than approximately 50%. Minocycline has a PIF1 of about 2.04, and an MPE of about 0.041.

The class of low phototoxicity tetracycline compound derivatives includes those derivatives having PIF 1 or PIF 2 values of approximately 1, i.e. 1 to about 2, preferably 1 to about 1.5. The class of low phototoxicity tetracycline derivatives optimally have MPE values of less than 0.1. Members of this class include, but are not limited to, tetracycline compounds having general formulae:

STRUCTURE K

wherein: R7, R8, and R9 taken together in each case, have the following meanings:

R7	R8	R9
hydrogen	hydrogen	amino
hydrogen	hydrogen	palmitamide
hydrogen	hydrogen	dimethylamino
trimethylammonium	hydrogen	hydrogen

and

STRUCTURE L **STRUCTURE M**

STRUCTURE N **STRUCTURE O**

wherein: R7, R8, and R9 taken together in each case, have the following meanings:

R7	R8	R9
hydrogen	hydrogen	acetamido
hydrogen	hydrogen	dimethylaminoacetamido
hydrogen	hydrogen	nitro
hydrogen	hydrogen	amino

and

STRUCTURE P

wherein: R8 and R9 taken together are, respectively, hydrogen and nitro.

The tetracycline compounds are preferably administered systemically or topically. For the purposes of this specification, "systemic administration" means administration to a human by a method that causes the compounds to be absorbed into the bloodstream.

For example, the tetracycline compounds can be administered orally by any method known in the art. For example, oral administration can be by tablets, capsules, pills, troches, elixirs, suspensions, syrups, wafers, chewing gum and the like.

Additionally, the tetracycline compounds can be administered enterally; parenterally, e.g., intravenously, intramuscularly, or subcutaneously, as injectable solutions or suspensions; intraperitoneally; or rectally. Administration can also be intranasally, in the form of, for example, an intranasal spray; or transdermally, in the form of, for example, a patch. For the treatment of asthma, administration by inhalation is preferred.

For the pharmaceutical purposes described above, the tetracycline compounds can be formulated in pharmaceutical preparations optionally with a suitable pharmaceutical carrier (vehicle) or excipient as understood by practitioners in the art. These preparations can be made according to conventional chemical methods.

In the case of tablets and capsules for oral use, carriers which are commonly used include lactose and corn starch. Lubricating agents such as magnesium stearate are commonly added. Further examples of carriers and excipients include milk, sugar, certain types of clay, gelatin, stearic acid or salts thereof, calcium stearate, talc, vegetable fats or oils, gums and glycols.

When aqueous suspensions are used for oral administration, emulsifying and/or suspending agents are commonly added. In addition, sweetening and/or flavoring agents may be added to the oral compositions.

For intramuscular, intraperitoneal, subcutaneous and intravenous use, sterile solutions of the tetracycline compounds can be employed. The pH of the solutions are preferably adjusted and buffered. For intravenous use, the total concentration of the solute(s) can be controlled in order to render the preparation isotonic.

The tetracycline compounds of the present invention optionally further comprise one or more additional pharmaceutically acceptable ingredient(s) such as alum, stabilizers, buffers, coloring agents, flavoring agents, and the like.

The tetracycline compound may be administered intermittently. For example, the tetracycline compound may be administered 1-6 times a day, preferably 1-4 times a day.

Alternatively, the tetracycline compound may be administered by sustained release. Sustained release administration is a method of drug delivery to achieve a certain level of the drug over a particular period of time. The level typically is measured by serum concentration. Further description of methods of delivering tetracycline compounds by sustained release can be found in U.S. Provisional Application No. 60/281,854, filed on April 5, 2001, and assigned to CollaGenex Pharmaceuticals, Inc. of Newtown, Pennsylvania. The aforementioned application is incorporated herein by reference in its entirety. For example, 40 milligrams of doxycycline may be administered by sustained release over a 24 hour period.

For topical application, the tetracycline compounds are placed in carrier compositions deemed to be suited for topical use, such as gels, salves, lotions, creams, ointments, ocular solutions, i.e. eye drops, and the like. The carrier compositions can also be incorporated into a support base or matrix which can be directly applied to the affected area. Examples of a support base or matrix include gauze or bandages.

The carrier compositions can comprise a tetracycline compound in amounts of up to about 25% (w/w). Amounts of from about 0.1% to about 10% are preferred.

Topical application is preferred for particular tetracycline compounds which have only limited biodistribution, e.g. CMT-5.

Combined or coordinated topical and systemic administration of the tetracycline compounds is also contemplated under the invention. For example, a systemically non-absorbable, non-antibiotic tetracycline compound can be

administered topically; while a tetracycline compound capable of substantial absorption and effective systemic distribution can be administered systemically.

The tetracycline compounds are prepared by methods known in the art. For example, natural tetracyclines may be modified without losing their antibiotic properties, although certain elements of the structure must be retained. The modifications that may and may not be made to the basic tetracycline structure have been reviewed by Mitscher in *The Chemistry of Tetracyclines*, Chapter 6, Marcel Dekker, Publishers, New York (1978). According to Mitscher, the substituents at positions 5-9 of the tetracycline ring system may be modified without the complete loss of antibiotic properties. Changes to the basic ring system or replacement of the substituents at positions 1-4 and 10-12, however, generally lead to synthetic tetracyclines with substantially less or effectively no antibiotic activity.

Further methods of preparing the tetracycline compounds are described in the examples.

EXAMPLES

The following examples serve to provide further appreciation of the invention but are not meant in any way to restrict the effective scope of the invention.

Preparation of Compounds

EXAMPLE 1

4-Dedimethylamino-7-dimethylamino-6-demethyl-6-deoxy-9-nitrotetracycline sulfate

To a solution of one millimole of 4-dedimethylamino-7-dimethylamino-6-demethyl-6-deoxytetracycline in 25 ml of concentrated sulfuric acid at 0°C was added 1.05 mmole of potassium nitrate. The resulting solution was stirred at ice bath temperature for 15 minutes and poured in one liter of cold ether with stirring. The

precipitated solid was allowed to settle and the majority of solvent decanted. The remaining material was filtered through a sintered glass funnel and the collected solid was washed well with cold ether. The product was dried in a vacuum desiccator overnight.

EXAMPLE 2

9-amino-4-dedimethylamino-7-dimethylamino-6-demethyl-6-deoxytetracycline sulfate

To a solution of 300 mg of the 9-nitro compound from example 1, in 30 ml of ethanol was added 50 mg of PtO_2. The mixture was hydrogenated at atmospheric pressure until the theoretical amount of hydrogen was absorbed. The system is flushed with nitrogen, the catalyst PtO_2 is filtered and the filtrate added dropwise to 300 ml of ether. The product that separates is filtered and dried in a vacuum desiccator.

EXAMPLE 3

9-Acetamido-4-dedimethylamino-7-dimethylamino-6-demethyl-6-deoxytetracycline sulfate

To a well stirred cold solution of 500 mg of 9-amino-4-dedimethylamino-7-dimethylamino-6-demethyl-6-deoxytetracycline sulfate from example 2, in 2.0 ml of 1.3-dimethyl-2-imidazolidinone, 500 mg of sodium bicarbonate was added followed by 0.21 ml of acetyl chloride. The mixture is stirred at room temperature for 30 minutes, filtered and the filtrate was added dropwise to 500 ml of ether. The product that separated was filtered and dried in a vacuum desiccator.

EXAMPLE 4

4-Dedimethylamino-7-dimethylamino-6-demethyl-6-deoxy-9-diazoniumtetracycline sulfate

To a solution of 0.5 g of 9-amino-4-dedimethylamino-7-dimethylamino-6-demethyl-6-deoxytetracycline sulfate, from example 2, in 10 ml of 0.1N hydrochloric acid in methanol cooled in an ice bath, 0.5 ml of n-butyl nitrite was added. The solution was stirred at ice bath temperature for 30 minutes and then poured into 250 ml of ether. The product that separated was filtered, washed with ether and dried in a vacuum desiccator.

EXAMPLE 5

9-Azido-4-dedimethylamino-7-dimethylamino-6-demethyl-6-deoxytetracycline sulfate

To a solution of 0.3 mmole of 4-dedimethylamino-7-dimethylamino-6-demethyl-6-deoxy-9-diazoniumtetracycline sulfate, from example 4, 10 ml of 0.1 N methanolic hydrogen chloride was added 0.33 mmole of sodium azide. The mixture was stirred at room temperature for 1.5 hours. The reaction mixture was then poured into 200 ml of ether. The product that separated was filtered and dried in a vacuum desiccator.

EXAMPLE 6

9-Amino-8-chloro-4-dedimethylamino-7-dimethylamino-6-demethyl-6-deoxy-tetracycline sulfate

One gram of 9-azido-4-dedimethylamino-7-dimethylamino-6-demethyl-6-deoxytetracycline hydrochloride, from example 4, was dissolved in 10 ml of concentrated sulfuric acid saturated with HCL at 0°C. The mixture was stirred at ice bath temperature for 1.5 hours and then slowly added dropwise to 500 ml of cold ether. The product that separated was filtered, washed with ether and dried in a vacuum desiccator.

EXAMPLE 7

4-Dedimethylamino-7-dimethylamino-6-demethyl-6-deoxy-9-ethoxythiocarbonylthio-tetracycline sulfate

A solution of 1.0 mmole of 4-dedimethylamino-7-dimethylamino-6-demethyl-6-deoxy-9-diazoniumtetracycline sulfate, from example 4, in 15 ml of water was added to a solution of 1.15 mmole of potassium ethyl xanthate in 15 ml of water. The mixture was stirred at room temperature for one hour. The product separated and was filtered and dried in a vacuum desiccator.

EXAMPLE 8A

General Procedure for Nitration

To 1 mmole of a 4-dedimethylamino-6-deoxytetracycline in 25 ml of concentrated sulfuric acid at 0°C was added 1 mmole of potassium nitrate with stirring. The reaction solution was stirred for 15 minutes and then poured into 100 g of chopped ice. The aqueous solution was extracted 5 times with 20 ml of butanol each time. The butanol extracts were washed three times with 10 ml of water each time, and concentrated *in vacuo* to a volume of 25 ml. The light yellow crystalline solid which precipitated was filtered, washed with 2 ml of butanol and dried *in vacuo* at 60°C for 2 hours. This solid was a mixture of the two mononitro isomers.

EXAMPLE 8B

4-Dedimethylamino-6-deoxy-9-nitrotetracycline

To 980 mg of the nitration product from 4-dedimethylamino-6-deoxytetracycline (a mixture of the 2 isomers) in 25 ml of methanol was added enough triethylamine to dissolve the solid. The filtered solution (pH 9.0) was adjusted to pH 5.2 with concentrated sulfuric acid. A crystalline yellow solid (236 mg.) was obtained (29% yield). The material at this point was quite pure and contained only small amounts of the 7-isomer. Final purification was accomplished by liquid partition chromatography using a diatomaceous earth packed column and the solvent system: chloroform: butanol: 0.5 *M* phosphate buffer (pH 2) (16:1:10).

EXAMPLE 9

4-Dedimethylamino-6-deoxy-7-nitrotetracycline

The methanol filtrate from example 8 was immediately adjusted to pH 1.0 with concentrated sulfuric acid. The light yellow crystalline solid, which was obtained as the sulfate salt. A purified free base was obtained by adjusting an aqueous solution of the sulfate salt (25 mg/ml) to pH 5.2 with 2 *N* sodium carbonate.

EXAMPLE 10

9-Amino-4-dedimethylamino-6-deoxytetracycline

To a solution of 300 mg of the 9-nitro compound, prepared in example 8, in 30 ml of ethanol was added 50 mg of PtO_2. The mixture was hydrogenated at atmospheric pressure until the theoretical amount of hydrogen was absorbed. The system is flushed with nitrogen, the PtO_2 catalyst is filtered and the filtrate added dropwise to 300 ml of ether. The solid that separates is filtered and dried in a vacuum desiccator.

EXAMPLE 11

9-Acetamido-4-dedimethylamino-6-deoxytetracycline sulfate

To well stirred cold solution of 500 mg of 9-amino-4-dedimethylamino-6-deoxytetracycline sulfate, from example 10, in 2.0 ml of 1,3-dimethyl-2-imidazolidinone was added 500 mg of sodium bicarbonate followed by 0.21 ml of acetyl chloride. The mixture was stirred at room temperature for 30 minutes, filtered and the filtrate was added dropwise to 500 ml of ether. The solid that separated was filtered and dried in a vacuum desiccator.

EXAMPLE 12

4-Dedimethylamino-6-deoxy-9-diazoniumtetracycline sulfate

To a solution of 0.5 g of 9-amino-4-dedimethylamino-6-deoxytetracycline sulfate, from example 10, in 10 ml of 0.1N hydrochloric acid in methanol cooled in an ice bath was added 0.5 ml of n-butyl nitrite. The solution was stirred at ice bath temperature for 30 minutes and the poured into 250 ml of ether. The solid that separated was filtered, washed with ether and dried in a vacuum desiccator.

EXAMPLE 13

9-Azido-4-dedimethylamino-6-deoxytetracycline sulfate

To a solution of 0.3 mmole of 4-dedimethylamino-6-deoxy-9-diazoniumtetracycline sulfate, of example 12, 10 ml of 0.1 N methanolic hydrogen chloride was added 0.33 mmole of sodium azide. The mixture was stirred at room temperature for 1.5 hours. The reaction mixture was then poured into 200 ml of ether. The solid that separated was filtered and dried in a vacuum desiccator.

EXAMPLE 14

9-Amino-8-chloro-4-dedimethylamino-6-deoxytetracycline sulfate

One gram of 9-azido-4-dedimethylamino-7-dimethylamino-6-deoxytetracycline hydrochloride, from example 13, was dissolved in 10 ml of concentrated sulfuric acid saturated with HCL at 0°C. The mixture was stirred at ice bath temperature for 1.5 hours and then slowly added dropwise to 500 ml of cold ether. The solid that separated was filtered, washed and ether and dried in a vacuum desiccator.

EXAMPLE 15

4-Dedimethylamino-6-deoxy-9-ethoxythiocarbonylthiotetracycline sulfate

A solution of 1.0 mmole of 4-dedimethylamino-6-deoxy-9-diazoniumtetracycline sulfate, from example 12, in 15 ml of water was added to a solution of 1.15 mmole of potassium ethyl xanthate in 15 ml of water. The mixture was stirred at room temperature for one hour. The solid that separated was filtered and dried in a vacuum desiccator.

EXAMPLE 16

9-Dimethylamino-4-dedimethylamino-6-deoxytetracycline sulfate

To a solution of 100 mg. of the 9-amino compound from example 10, in 10 ml of ethylene glycol monomethyl ether is added 0.05 ml of concentrated sulfuric acid, 0.4 ml. of a 40% aqueous formaldehyde solution and 100 mg of a 10% palladium on carbon catalyst. The mixture is hydrogenated under atmospheric pressure and room temperature for 20 minutes. The catalyst was filtered and the filtrate was evaporated to dryness under reduced pressure. The residue is dissolved in 5 ml of methanol and this solution was added to 100 ml of ether. The product that separated was filtered and dried, yield, 98 mg.

EXAMPLE 17

7-Amino-4-dedimethylamino-6-deoxytetracycline

This compound can be made using Procedure A or B. Procedure A. To a solution of 300 mg of the 7-nitro compound, from example 1, in 30 ml of ethanol was added 50 mg of PtO_2. The mixture was hydrogenated at atmospheric pressure until the theoretical amount of hydrogen was absorbed. The system is flushed with nitrogen, the catalyst PtO_2 is filtered and the filtrate added dropwise to 300 ml of ether. The solid that separates is filtered and dried in a vacuum desiccator.

Procedure B. 1 g of 6-deoxy-4-dedimethylamino-tetracycline was dissolved in 7.6 ml THF and 10.4 ml methanesulfonic acid at -10°C. After warming the mixture to 0°C a solution of 0.86 g of dibenzyl azodicarboxylate was added and the mixture stirred for 2 hours at 0°C to yield 7-[1,2-bis(carbobenzyloxy)hydrazino]-4-dedimethylamino-6-deoxytetracycline. A solution of 1 millimole of this material in 70 ml 2-methoxyethanol, and 300 mg 10% Pd-C was hydrogenated at room temperature to give 7-amino-6-deoxy-4-dedimethylaminotetracycline.

EXAMPLE 18

7-Amino-6-deoxy-5-hydroxy-4-dedimethylaminotetracycline

1g of 6-deoxy-5-hydroxy-4-dedimethylaminotetracycline 3 was dissolved in 7.6 ml THF and 10.4 ml methanesulfonic acid at -10°C. After warming the mixture to 0°C a solution of 0.86g dibenzyl azodicarboxylate in 0.5 ml THF was added and the mixture stirred for 2 hours at 0°C to yield 7-[1,2-bis(carbobenzyloxy)hydrazino]-4-dedimethylamino-6-deoxy-5-hydroxytetracycline. A solution of 1 millimole of this material in 70 ml 2-methoxyethanol, and 300 mg 10% Pd-C was hydrogenated at room temperature to give 7-amino-6-deoxy-5-hydroxytetracycline.

EXAMPLE 19

7-Acetamido-4-dedimethylamino-6-deoxy-5-hydroxytetracycline sulfate.

To well stirred cold solution of 500 mg of 7-amino-4-dedimethylamino-6-deoxy-5-hydroxytetracycline sulfate, from example 18, in 2.0 ml of 1,3-dimethyl-2-imidazolidinone was added 500 mg of sodium bicarbonate followed by 0.21 ml of acetyl chloride. The mixture was stirred at room temperature for 30 minutes, filtered and the filtrate was added dropwise to 500 ml of ether. The solid that separated was filtered and dried in a vacuum desiccator.

EXAMPLE 20

4-Dedimethylamino-6-deoxy-5-hydroxy-7-diazoniumtetracycline hydrochloride

To a solution of 0.5 g of 7-amino-4-dedimethylamino-6-deoxy-5-hydroxytetracycline sulfate, from example 20, in 10 ml of 0.1N hydrochloric acid in methanol cooled in an ice bath was added 0.5 ml of n-butyl nitrite. The solution was stirred at ice bath temperature for 30 minutes and then poured into 250 ml of ether. The solid that separated was filtered, washed with ether and dried in a vacuum desiccator.

EXAMPLE 21

7-Azido-4-dedimethylamino-6-deoxy-5-hydroxytetracycline

To a solution of 0.3 mmole of 4-dedimethylamino-6-deoxy-5-hydroxy-7-diazoniumtetracycline hydrochloride, from example 20, 10 ml of 0.1 N methanolic hydrogen chloride was added 0.33 mmole of sodium azide. The mixture was stirred at room temperature for 1.5 hours. The reaction mixture was then poured into 200 ml of ether. The solid that separated was filtered and dried in a vacuum desiccator.

EXAMPLE 22

7-Amino-8-chloro-4-dedimethylamino-6-deoxy-5-hydroxytetracycline sulfate

One gram of 7-azido-4-dedimethylamino-7-dimethylamino-6-deoxy-5-hydroxytetracycline sulfate, from example 21, was dissolved in 10 ml of concentrated sulfuric acid (previously saturated with hydrogen chloride) at 0°C. The mixture was stirred at ice bath temperature for 1.5 hours and then slowly added dropwise to 500 ml of cold ether. The solid that separated was filtered, washed with ether and dried in a vacuum desiccator.

EXAMPLE 23

4-Dedimethylamino-6-deoxy-5-hydroxy-7-ethoxythiocarbonylthiotetracycline

A solution of 1.0 mmole of 4-dedimethylamino-6-deoxy-5-hydroxy-7-diazoniumtetracycline hydrochloride, from example 20, in 15 ml of water was added to a solution of 1.15 mmole of potassium ethyl xanthate in 15 ml of water. The mixture was stirred at room temperature for one hour. The solid that separated was filtered and dried in a vacuum desiccator.

EXAMPLE 24

7-Dimethylamino-4-dedimethylamino-6-deoxy-5-hydroxytetracycline sulfate

To a solution of 100 mg of the 7-amino compound in 10 ml of ethylene glycol monomethyl ether is added 0.05 ml of concentrated sulfuric acid, 0.4 ml of a 40% aqueous formaldehyde solution and 100 mg of a 10% palladium on carbon catalyst. The mixture is reduced with hydrogen at atmospheric pressure and room temperature for 20 minutes. The catalyst was filtered and the filtrate was evaporated to dryness under reduced pressure. The residue is dissolved in 5 ml of methanol and this solution was added to 100 ml of ether. The product that separated was filtered and dried, yield, 78 mg.

EXAMPLE 25

7-Diethylamino-4-dedimethylamino-5-hydroxytetracycline sulfate

To a solution of 100 mg of the 7-amino compound in 10 ml of ethylene glycol monomethyl ether is added 0.05 ml of concentrated sulfuric acid, 0.4 ml of acetaldehyde and 100 mg of a 10% palladium on carbon catalyst. The mixture is reduced with hydrogen at atmospheric pressure at room temperature for 20 minutes. The catalyst was filtered and filtrate was evaporated to dryness under reduced pressure. The residue is dissolved in 5 ml of methanol and this solution was added to 100 ml of ether. The product that separated was filtered and dried.

EXAMPLE 26

4-Dedimethylamino-6-deoxy-7-diazoniumtetracycline hydrochloride

To a solution of 0.5 g. of 7-amino-4-dedimethylamino-6-deoxytetracycline sulfate, from example 17, in 10 ml of 0.1N hydrochloric acid in methanol cooled in an ice bath was added 0.5 ml of n-butyl nitrite. The solution was stirred at ice bath temperature for 30 minutes and then poured into 250 ml of ether. The solid that separated was filtered, washed with ether and dried in a vacuum desiccator.

EXAMPLE 27

7-Azido-4-dedimethylamino-6-deoxytetracycline

To a solution of 0.3 mmole of 4-dedimethylamino-6-deoxy-7-diazoniumtetracycline hydrochloride, from example 26, 10 ml of 0.1 N methanolic hydrogen chloride was added 0.33 mmole of sodium azide. The mixture was stirred at room temperature for 1.5 hours. The reaction mixture was then poured into 200 ml of ether. The solid that separated was filtered and dried in a vacuum desiccator.

EXAMPLE 28

7-Amino-8-chloro-4-dedimethylamino-6-deoxytetracycline sulfate

One gram of 7-azido-4-dedimethylamino-7-dimethylamino-6-deoxytetracycline sulfate was dissolved in 10 ml of concentrated sulfuric acid (previously saturated with hydrogen chloride) at 0°C. The mixture was stirred at ice bath temperature for 1.5 hours and then slowly added dropwise to 500 ml of cold ether. The solid that separated was filtered, washed with ether and dried in a vacuum desiccator.

EXAMPLE 29

4-Dedimethylamino-6-deoxy-7-ethoxythiocarbonylthiotetracycline

A solution of 1.0 mmole of 4-dedimethylamino-6-deoxy-7-diazoniumtetracycline hydrochloride, from example 26, in 15 ml of water was added to a solution of 1.15 mmole of potassium ethyl xanthate in 15 ml of water. The mixture was stirred at room temperature for one hour. The solid that separated was filtered and dried in a vacuum desiccator.

EXAMPLE 30

7-Dimethylamino-4-dedimethylamino-6-deoxytetracycline sulfate

To a solution of 100 mg of the 7-amino compound, from example 26, in 10 ml of ethylene glycol monomethyl ether is added 0.05 ml of concentrated sulfuric acid, 0.4 ml of a 40% aqueous formaldehyde solution and 100 mg of a 10% palladium on carbon catalyst. The mixture is reduced with hydrogen at atmospheric pressure and room temperature for 20 minutes. The catalyst was filtered and the filtrate was evaporated to dryness under reduced pressure. The residue is dissolved in 5 ml of methanol and this solution was added to 100 ml of ether. The product that separated was filtered and dried.

EXAMPLE 31

9-Acetamido-8-chloro-4-dedimethylamino-7-dimethylamino-6-deoxy-6-demethyltetracycline

To well stirred cold solution of 500 mg of 9-amino-8-chloro-4-dedimethylamino-6-deoxy-6-demethyl-7-dimethyl amino tetracycline sulfate, from example 6, in 2.0 ml of 1,3-dimethyl -2-imidazolidinone was added 500 mg of sodium bicarbonate followed by 0.21 ml. of acetyl chloride. The mixture was stirred at room temperature for 30 minutes, filtered and the filtrate was added dropwise to 500 ml of ether. The solid that separated was filtered and dried in a vacuum desiccator.

EXAMPLE 32

8-Chloro-4-dedimethylamino-7-dimethylamino-6-deoxy-6-demethyl-9-ethoxythiocarbonylthiotetracycline

A solution of 1.0 mmole of -8-chloro-4-dedimethylamino-6-deoxy-6-demethyl-7-dimethyl amino-9-diazoniumtetracycline hydrochloride in 15 ml of water was added to a solution of 1.15 mmole of potassium ethyl xanthate in 15 ml of water. The mixture was stirred at room temperature for one hour. The solid that separated was filtered and dried in a vacuum desiccator.

EXAMPLE 33

8-Chloro-9-dimethylamino-4-dedimethylamino-7-dimethylamino-6-deoxy-6-demethytetracycline sulfate

To a solution of 100 mg. of the 9- amino compound, from example 6, in 10 ml of ethylene glycol monomethyl ether is added 0.05 ml of concentrated sulfuric acid, 0.4 ml of acetaldehyde and 100 mg of a 10% palladium on carbon catalyst. The mixture is reduced with hydrogen at atmospheric pressure and room temperature for 20 minutes. The catalyst was filtered and the filtrate was evaporated to dryness under reduced pressure. The residue is dissolved in 5 ml of methanol and this solution was added to 100 ml of ether. The product that separated was filtered and dried.

EXAMPLE 34

N-(4-methylpiperazin-1-yl) methyl-4-dedimethylamino-6-demethyl-6-deoxytetracycline

An aqueous solution of 58 mg (37%) formaldehyde (0.72 mmol) was added to a solution of 203 mg (0.49 mmol) of 4-dedimethylamino-6-demethyl-6-deoxytetracycline in 5.0 ml ethylene glycol dimethyl ether. The mixture was stirred at room temperature for 0.5 hours. 56 mg (0.56 mmol) of 1-methylpiperazine was then added and the resulting mixture was stirred overnight and refluxed for 20 minutes. The mixture was then cooled and a solid product was collected by filtration. The solid product was then washed with the solvent and dried by vacuum filtration.

EXAMPLE 35

N-(4-methylpiperazin-1-yl)methyl-4-dedimethylamino-6-demethyl-6-deoxy-9-hexanoylaminotetracycline

An aqueous solution of 49 mg 37 % formaldehyde (0.60 mmol) was added to a solution of 146 mg (0.30 mmol) of 4-dedimethylamino-6-demethyl-6-deoxy-9-hexanoylaminotetracycline in 5.0 ml ethylene glycol dimethyl ether. The mixture was stirred at room temperature for 0.5 hours. 60 mg (0.60 mmol) of 1-methylpiperazine was then added and the resulting mixture was stirred overnight and refluxed for 20 minutes. The mixture was then cooled and a solid product was collected by filtration. The solid product was then washed with the solvent and dried by vacuum filtration.

EXAMPLE 36

4-Dedimethylamino-6-demethyl-6-deoxy-9-hexanoylaminotetracycline.

1.54 g (7.2 mmol) of hexanoic anhydride and 150 mg of 10% Pd/C catalyst were added to 300 mg (0.72 mmol) of 4-dedimethylamino-6-demethyl-6-deoxytetracycline in 6.0 ml of 1,4-dioxane and 6.0 ml of methanol. The mixture was

hydrogenated overnight at room temperature. The catalyst was removed by filtration and the filtrate was concentrated under reduced pressure. The residue was dissolved in 7 ml of ethyl acetate and trituated with 50 ml of hexane to produce a solid product. The solid product was filtered and dried by vacuum filtration.

EXAMPLE 37

Phototoxicity Determination

BALB/c 3T3 (CCL-163) cells were obtained from ATCC and cultured in antibiotic-free Dulbecco's Minimum Essential Medium (4.5 g/1 glucose)(DMEM) supplemented with L-glutamine (4mM) and 10% newborn calf serum. The working cell bank was prepared and found to be free of mycoplasma. Streptomycin sulfate (100g/ml) and penicillin (100 IU/ml) were added to the medium after the cells were treated with test article in 96-well plates.

Serial dilutions of the tetracycline derivatives were prepared in DMSO at concentrations 100x to final testing concentration. The COL dilutions in DMSO were then diluted in Hanks' Balanced Salt Solution (HBSS) for application to the cells. The final DMSO concentration was 1% in treated and control cultures. A dose range finding assay is conducted with eight serial dilutions covering a range of 100-0.03 μg/ml in half log steps. Definitive assays are conducted with 6-8 serial dilutions prepared in quarter log steps, centered on the expected 50% toxicity point as determined in the dose range finding assay. One hundred 100 μg/ml was the highest dose recommended to prevent false negative results from UV absorption by the dosing solutions. One dose range finding and at least two definitive trials were performed on each tetracycline derivative and control compound.

Controls: Each assay included both negative (solvent) and positive controls. Twelve wells of negative control cultures were used on each 96-well plate. Chlorpromazine (Sigma Chemicals) was used as the positive control and was prepared and dosed like the test tetracycline derivatives.

Solar Simulator: A Dermalight SOL 3 solar simulator, equipped with a UVA H1 filter (320-400 nm), was adjusted to the appropriate height. Measurement of energy through the lid of a 96-well microtiter plate was carried out using a calibrated UV radiometer UVA sensor. Simulator height was adjusted to deliver 1.7 ± 0.1 mW/cm^2 of UVA energy (resulting dose was 1 J/cm^2 per 10 minutes of exposure).

Phototoxicity Assay: Duplicate plates were prepared for each test material by seeding 10^4 3T3 cells per well in complete medium 24 hours before treatment. Prior to treatment, the medium was removed, and the cells washed once with 125 µl of prewarmed HBSS. Fifty µl of prewarmed HBSS were added to each well. Fifty µl of each test article dilution were added to the appropriate wells and the plates returned to the incubator for approximately one hour. Six wells were treated with each dose of test or control article on each plate. Following the 1 hr incubation, the plates designated for the photo irradiation were exposed (with the lid on) to 1.7 ± 0.1 mW/cm^2 UVA light for 50 ±2 minutes at room temperature resulting in an irradiation dose of 5 J/cm^2. Duplicate plates, designated for the measurement of cytotoxicity without light, were kept in the dark room temperature for 50 ± 2 minutes. After the 50 minute exposure period (with or without light) the test article dilutions were decanted from the plates and the cells washed once with 125 µl of HBSS. One hundred µl of medium were added to all wells and the cells incubated as above for 24 ± 1 hours.

After 24 hours of incubation, the medium was decanted and 100 µl of the Neutral Red containing medium were added to each well. The plates were returned to the incubator and incubated for approximately 3 hours. After 3 hours, the medium was decanted and each well rinsed once with 250 µl of HBSS. The plates were blotted to remove the HBSS and 100 µl of Neutral Red Solvent were added to each well. After a minimum of 20 minutes of incubation at room temperature (with shaking), the absorbance at 550 nm was measured with a plate reader, using the mean of the blank outer wells as the reference. Relative survival was obtained by comparing the amount of neutral red taken by each well treated with the test article and positive

control to the neutral red taken up by the average of the negative wells (12 wells) on the same plate. The amount of neutral red taken up by the negative control wells is considered to be 100% survival.

There are several methods by which to quantify relative phototoxicity, e.g., the photoirritancy factor (PIF) and the mean photo effect (MPE), as discussed below.

Phototoxicity Determined by PIF Valuations

To determine the dose where there is a 50% decrease in relative viability, the relative cell viability is plotted as a function of increasing dose and a polynomial equation is calculated to produce the "best fit" line through all the points. The dose of a test substance corresponding to the point where this line crosses the 50% survival point is calculated (termed the Inhibitory Concentration 50% or IC_{50}) and used to compare the toxicity of the test chemical in the presence and absence of UVA/visible light.

Phototoxicity of a tetracycline derivative can be measured by its photoirritancy factor (PIF). The photo-irritancy factor (PIF) is the ratio of the IC_{50}, value in the absence of light to the IC_{50} value in the presence of light. That is, the PIF was determined by comparing the IC_{50} without UVA [IC_{50}(-UVA)] with the IC_{50} with UVA [IC_{50}(+UVA)]:

$$PIF = \frac{IC_{50}(-UVA)}{IC_{50}(+UVA)}$$

IC_{50} values for both the UVA exposed and non-exposed groups were determined whenever possible. If the two values are the same, the PIF is 1 and there is no phototoxic effect. If the action of the light increases toxicity, the IC_{50} with light will be lower than the IC_{50} without light, and the PIF will increase.

If IC_{50} (+UVA) can be determined but IC_{50}(-UVA) cannot, the PIF cannot be calculated, although the compound tested may have some level of phototoxic potential. In this case, a ">PIF" can be calculated and the highest testable dose (-UVA) will be used for calculation of the ">PIF."

$$>PIF = \frac{\text{maximum dose (-UVA)}}{IC_{50}(+UVA)}$$

If both, IC_{50}(-UVA) and IC_{50}(+UVA) cannot be calculated because the chemical does not show cytotoxicty (50% reduction in viability) up to the highest dose tested, this would indicate a lack of phototoxic potential.

In calculating PIF values, the data resulting from the assay procedure can be interpreted by different methods.

For example, during the period March 2, 1999 to April 16, 1999, PIF values were obtained using the earlier phototoxicity software and its curve-fitting algorithms, i.e. PIF1.

Since April 1999, the 3T3 phototoxicity assay has undergone extensive validation, and has now been incorporated into a draft guideline by the Organization of Economic Cooperation and Development (OECD) (Draft Guideline 432). (See Spielmann et al., The International EU/COLIPA *In Vitro* Phototoxicity Validation Study; Results of Phase II (blind trial). Part 1: The 3T3 NRU Phototoxicity Test. Toxicology *In Vitro* 12:305-327 (1998); and Spielmann et al., A Study on UV Filter Chemicals from Annex VII of European Union Directive 76/768/EEC, in the *In Vitro* 3T3 Phototoxicity Test. ATLA 26:679-708 (1998).) The new guideline follows the same assay procedure, but provides some additional guidance in the interpretation of the resulting data, and incorporates updated software. As used herein, the PIF value interpreted by this method is termed PIF2.

According to this updated OECD draft guideline, the IC_{50} values are developed from curves fitted to the data by a multiple boot strap algorithm. The curve fitting and calculations of the PIF are performed by software developed under contract to the German government (ZEBET, Berlin).

In particular, since there are six wells (and therefore six relative survival values) for each dose, the software performs multiple calculations of the best fit line using what is called boot strapping. This approach is used to account for variations in the data. From the bootstrapped curves, the software determines a mean IC_{50} for the treatment. The IC_{50} is used to compare the toxicity of the test chemical in the presence and absence of UVA/visible light. Figure 2 shows an example of a set of dose response curves prepared for the positive control chemical Chlorpromazine. The difference in the IC_{50} values can be clearly seen in this example of a highly phototoxic chemical.

Using the original software and evaluation procedures, if both IC_{50} values can be determined, the cut off value of the factor to discriminate between phototoxicants and non-phototoxicants is a factor of 5. A factor greater than 5 is indicative of phototoxic potential of the test material. Using this software, the mean PIF1 for COL 10 was determined to be 1.83. The mean PIF1 for COL 1002 was determined to be 1.12.

The OECD draft guideline has revised the values for the PIF used to differentiate between phototoxins, potential phototoxins and non-phototoxins. A PIF2 of less than 2 is considered non-phototoxic, 2 to less than 5 is considered potentially phototoxic, and 5 or greater is considered clearly phototoxic. In accordance with the OECD draft guideline, the mean PIF2 values of COL 10 and COL 1002 are 2.04 and 1.35, respectively.

Phototoxicity Determined by MPE Valuations

At each data point, a photo effect is calculated according to the following formula:

$$\text{Photo Effect}_c = \text{Dose Effect}_c \times \text{Response Effect}_c \qquad (\text{i.e., } PE_c = DE_c \times RE_c)$$

where **c** represents one concentration

Dose Effect$_c$ compares the dose required to achieve percent survival **n** without UVA (c) with the dose required to achieve the same percent survival with UVA (c'):

$$\text{Dose Effect}_n = \frac{(\text{Dose (-UVA) to give survival } \mathbf{n} \,/\, \text{Dose (+UVA) to give survival n}) - 1}{(\text{Dose (-UVA) to give survival } \mathbf{n} \,/\, \text{Dose (+UVA) to give survival n}) + 1}$$

As the ratio increases, the Dose Effect term approaches 1.

In the example in Figure 3, the Dose Effect is calculated for one point. The dose of 0.4 dose units is required to reduce cell viability (termed **response** on the y axis) to 66% in the absence of light while only 0.16 dose units are required to similarly reduce viability in the presence of light. The dose effect for 0.4 dose units is:

$$DE_{0.4} = \frac{|\,(0.4/0.16) - 1\,|}{|\,(0.4/0.16) + 1\,|} = 0.43$$

The Response Effect at dose c compares the percent survival with and without UVA at that dose and normalizes for the total range of the response over the range of doses evaluated (n_1 to n_i).

$$\text{Response Effect}_c = \frac{R(\text{-UVA})c - R(\text{+UVA})c}{R_0}$$

where R_0 is the Total Survival Range (up to 100%), R(-UVA)c is the survival without UVA at dose c, and R(+UVA)c is the survival with UVA at dose c.

As the difference between the survival without UVA at dose c and the survival with UVA at dose c [ie., R(-UVA)c - R(+UVA)c] increases (indicative of phototoxic potential), then the Response $Effect_c$ approaches 1.0.

Again in Figure 3, the Response Effect for the 0.4 dose is :

$RE_{0.4} = (66\% - 11\%) / 100\% = 0.55$

The PE in this example is $PE_{0.4} = 0.43 * 0.55 = 0.24$

The Mean Photo Effect is the mean of the individual Photo Effect values over the range evaluated. It is produced from the formula:

$$MPE = \frac{\sum_{i=1}^{n} w_i * PE_{ci}}{\sum_{i=1}^{n} w_i}$$

where w_i is a weighting factor for the highest viability observed for each curve.

The MPE value is used to determine phototoxic potential. In the original analysis of the validation data, a material was considered nonphototoxic if the MPE was < 0.1 (this includes negative MPE values) and phototoxic if the MPE was ≥ 0.1 (Spielmann et al, 1998). This cut off was re-examined once the software had been rewritten and the weighting factor added. In the draft Organization for Economic Cooperation and Development phototoxicity test guideline (Guideline 432), MPE values of < 0.1 (including negative values) are considered indicative of a nonphototoxin, values of 0.1 to <0.15 are considered probable phototoxins, and greater than and equal to 0.15 clear phototoxins. This guideline is expected to become the standard after final approval in 2003. The software used to calculate the MPE values is part of this guideline.

Thus, while there have been described what are presently believed to be the preferred embodiments of the present invention, those skilled in the art will realize that other and further embodiments can be made without departing from the spirit of the invention, and it is intended to include all such further modifications and changes as come within the true scope of the claims set forth herein.

The following table shows the phototoxicity values for several tetracycline derivatives. The positive control is chlorpromazine. The phototoxicity is evaluated in terms of MPE and in terms of PIF using the new OECD draft guideline.

PHOTOTOXICITY VALUES

COMPOUND	MPE	PIF 1	PIF 2
Chlorpromazine	0.639	N/D	40.38
Tetracycline	0.340	5.38	N/A
Doxycycline	0.522	23.37	26.71
Minocycline	0.041	2.04	N/A
COL 10	0.099	1.82	2.04
COL 1	0.460	N/D	N/A
COL 2	0.005	N/D	N/A
COL 3	0.654	647	84.72
COL 302	0.378	23.16	23.32
COL 303	0.309	5.27	13.82
COL 305	0.420	N/D	N/A
COL 306	0.038	1.64	1.56
COL 307	0.056	1.17	N/A
COL 308	0.015	1.0	N/A
COL 309	0.170	5.17	12.87
COL 311	0.013	1.0	N/A
COL 312	0.442	62.67	75.11
COL 313	0.462	80.27	58.22
COL 314	0.475	41.1	89.48
COL 315	0.276	15.8	35.30
COL 4	0.570	N/D	N/A
COL 5	0.186	N/D	N/A
COL 6	0.155	N/D	N/A
COL 7	0.531	N/D	N/A
COL 8	0.703	165	82.61
COL 801	-0.001	1.0	N/A
COL 802	-0.123	1.0	N/A
COL 803	0.047	N/D	N/A
COL 804	0.003	1.0	N/A
COL 805	0.022	1.0	N/A
COL 807	0.382	40.4	N/A
COL 808	0.387	46.45	N/A
COL 809	0.420	N/D	N/A
COL 9	0.546	N/D	N/A
COL 1001	0.025	N/D	N/A
COL 1002	0.040	1.0	1.35

5 N/A indicates that the IC_{50} value could not be determined for the UVA exposed and/or non-exposed groups

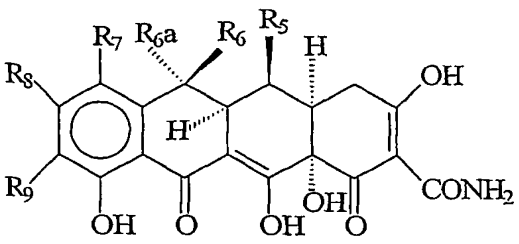
N/D indicates that the PIF1 was not determined for the particular compound, or was N/A as defined above.

In the present specification, some of the compounds of the invention are referred to by codes names. The correspondence between the compound and codes names are as follows:

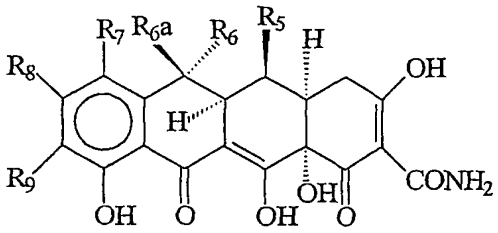
CHEMICAL NAMES OF THE COL COMPOUNDS

COL-1	4-dedimethylaminotetracycline
COL-3	6-demethyl-6-deoxy-4-dedimethylaminotetracycline
COL-301	7-bromo-6-demethyl-6-deoxy-4-dedimethylaminotetracycline
COL-302	7-nitro-6-demethyl-6-deoxy-4-dedimethylaminotetracycline
COL-303	9-nitro-6-demethyl-6-deoxy-4-dedimethylaminotetracycline
COL-304	7-acetamido-6-demethyl-6-deoxy-4-dedimethylaminotetracycline
COL-305	9-acetamido-6-demethyl-6-deoxy-4-dedimethylaminotetracycline
COL-306	9-dimethylamino-6-demethyl-6-deoxy-4-dedimethylaminotetracycline
COL-307	7-amino-6-demethyl-6-deoxy-4-dedimethylaminotetracycline
COL-308	9-amino-6-demethyl-6-deoxy-4-dedimethylaminotetracycline
COL-309	9-dimethylaminoacetamido-6-demethyl-6-deoxy-4-dedimethylaminotetracycline
COL-310	7-dimethylamino-6-demethyl-6-deoxy-4-dedimethylaminotetracycline
COL-311	9-palmitamide-6-demethyl-6-deoxy-4-dedimethylaminotetracycline
COL-312	2-$CONHCH_2$-pyrrolidin-1-yl-6-demethyl-6-deoxy-4-dedimethylaminotetracycline
COL-313	2-$CONHCH_2$-piperidin-1-yl-6-demethyl-6-deoxy-4-dedimethylaminotetracycline
COL-314	2-$CONHCH_2$-morpholin-1-yl-6-demethyl-6-deoxy-4-dedimethylaminotetracycline
COL-315	2-$CONHCH_2$-piperazin-1-yl-6-demethyl-6-deoxy-4-dedimethylaminotetracycline
COL-4	7-chloro-4-dedimethylaminotetracycline
COL-5	tetracycline pyrazole
COL-6	4-hydroxy-4-dedimethylaminotetracycline
COL-7	4-dedimethylamino-12α-deoxytetracycline
COL-8	4-dedimethylaminodoxycycline
COL-801	9-acetamido-4-dedimethylaminodoxycycline
COL-802	9-dimethylaminoacetamido-4-dedimethylaminodoxycycline
COL-803	9-palmitamide-4-dedimethylaminodoxycycline
COL-804	9-nitro-4-dedimethylaminodoxycycline
COL-805	9-amino-4-dedimethylaminodoxycycline
COL-806	9-dimethylamino-4-dedimethylaminodoxycycline
COL-807	2-$CONHCH_2$-pyrrolidin-1-yl-4-dedimethylaminodoxycycline
COL-808	2-$CONHCH_2$-piperidin-1-yl-4-dedimethylaminodoxycycline
COL-809	2-$CONHCH_2$-piperazin-1-yl-4-dedimethylaminodoxycycline
COL-10	4-dedimethylaminominocycline (a.k.a. COL-310)
COL-1001	7-trimethylammonium-4-dedimethylaminosancycline
COL-1002	9-nitro-4-dedimethylaminominocycline

INDEX OF STRUCTURES



Structure C



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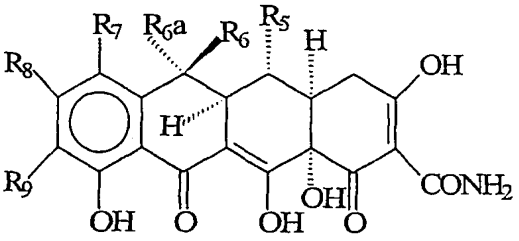

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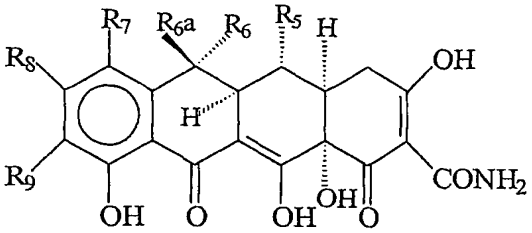
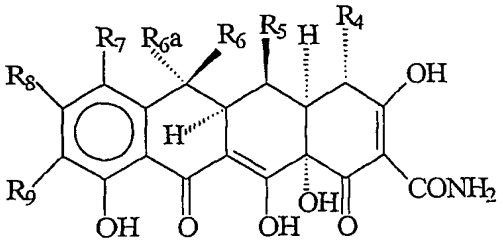


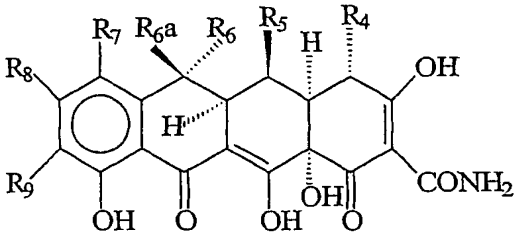
Structure F

wherein R7 is selected from the group consisting of hydrogen, amino, nitro, mono(lower alkyl) amino, halogen, di(lower alkyl)amino, ethoxythiocarbonylthio, azido, acylamino, diazonium, cyano, and hydroxyl; R6-a is selected from the group consisting of hydrogen and methyl; R6 and R5 are selected from the group consisting of hydrogen and hydroxyl; R8 is selected from the group consisting of hydrogen and halogen; R9 is selected from the group consisting of hydrogen, amino, azido, nitro, acylamino, hydroxy, ethoxythiocarbonylthio, mono(lower alkyl)amino, halogen, diazonium, di(lower alkyl)amino and $RCH(NH_2)CO$; R is hydrogen or lower alkyl; and pharmaceutically acceptable and unacceptable salts thereof; with the following provisos: when either R7 and R9 are hydrogen then R8 must be halogen; and when R6-a, R6, R5 and R9 are all hydrogen and R7 is hydrogen, amino, nitro, halogen, dimethylamino or diethylamino, then R8 must be halogen; and when R6-a is methyl, R6 and R9 are both hydrogen, R5 is hydroxyl and R7 is hydrogen, amino, nitro, halogen or diethylamino, then R8 is halogen; and when R6-a is methyl, R6 is

hydroxyl, R5, R7 and R9 are all hydrogen, then R8 must be halogen; and when R6-a, R6 and R5 are all hydrogen, R9 is methylamino and R7 is dimethylamino, then R8 must be halogen; and when R6-a is methyl, R6 is hydrogen, R5 is hydroxyl, R9 is methylamino and R7 is dimethylamino, then R8 must be halogen; and when R6-a is methyl, R6, R5 and R9 are all hydrogen and R7 is cyano, then R8 must be halogen.



Structure G



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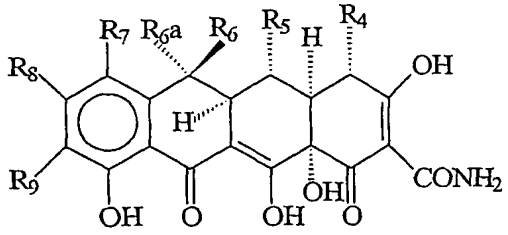
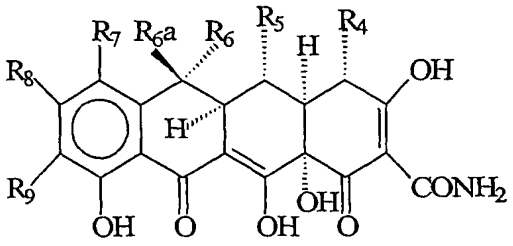


Structure I

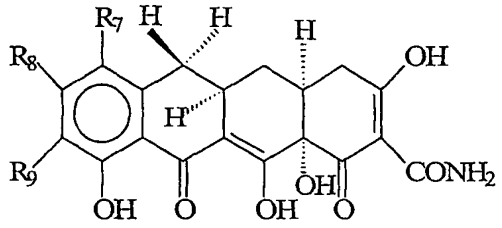


Structure J

wherein R7 is selected from the group consisting of hydrogen, amino, nitro, mono(lower alkyl) amino, halogen, di(lower alkyl)amino, ethoxythiocarbonylthio, azido, acylamino, diazonium, cyano, and hydroxyl; R6-a is selected from the group consisting of hydrogen and methyl; R6 and R5 are selected from the group consisting of hydrogen and hydroxyl; R4 is selected from the group consisting of NOH, N-NH-A, and NH-A, where A is a lower alkyl group; R8 is selected from the group consisting of hydrogen and halogen; R9 is selected from the group consisting of hydrogen, amino, azido, nitro, acylamino, hydroxy, ethoxythiocarbonylthio, mono(lower alkyl) amino, halogen, di(lower alkyl)amino and $RCH(NH_2)CO$; R is hydrogen or lower alkyl; and pharmaceutically acceptable and unacceptable salts thereof; with the following provisos: when R4 is NOH, N-NH-alkyl or NH-alkyl and R7, R6-a, R6, R5, and R9 are all hydrogen, then R8 must be halogen; and when R4 is

NOH, R6-a is methyl, R6 is hydrogen or hydroxyl, R7 is halogen, R5 and R9 are both hydrogen, then R8 must be halogen; and when R4 is N-NH-alkyl, R6-a is methyl, R6 is hydroxyl and R7, R5, R9 are all hydrogen, then R8 must be halogen; and when R4 is NH-alkyl, R6-a, R6, R5 and R9 are all hydrogen, R7 is hydrogen, amino, mono(lower alkyl)amino, halogen, di(lower alkyl)amino or hydroxyl, then R8 must be halogen; and when R4 is NH-alkyl, R6-a is methyl, R6 and R9 are both hydrogen, R5 is hydroxyl, and R7 is mono(lower alkyl)amino or di(lower alkyl)amino, then R8 must be halogen; and when R4 is NH-alkyl, R6-a is methyl, R6 is hydroxy or hydrogen and R7, R5, and R9 are all be hydrogen, then R8 must be halogen.

General Formula (I)



Structure K

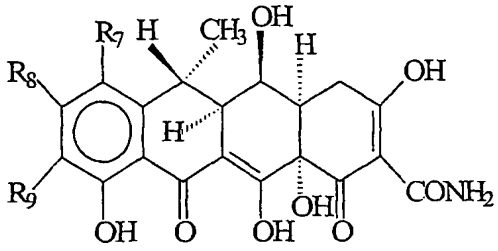
wherein R7, R8, and R9 taken together in each case, have the following meanings:

R7	R8	R9
azido	hydrogen	hydrogen
dimethylamino	hydrogen	azido
hydrogen	hydrogen	amino
hydrogen	hydrogen	azido
hydrogen	hydrogen	nitro
dimethylamino	hydrogen	amino
acylamino	hydrogen	hydrogen
hydrogen	hydrogen	acylamino
amino	hydrogen	nitro
hydrogen	hydrogen	(N,N-dimethyl)glycylamino
amino	hydrogen	amino
hydrogen	hydrogen	ethoxythiocarbonylthio
dimethylamino	hydrogen	acylamino

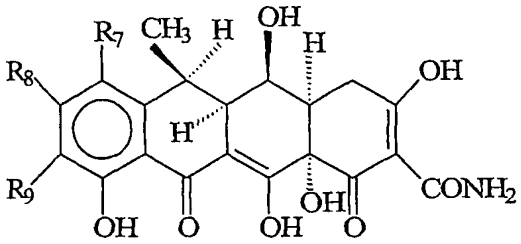
	dimethylamino	hydrogen	diazonium
	dimethylamino	chloro	amino
	hydrogen	chloro	amino
	amino	chloro	amino
	acylamino	chloro	acylamino
	amino	chloro	hydrogen
	acylamino	chloro	hydrogen
	monoalkylamino	chloro	amino
	nitro	chloro	amino
	dimethylamino	chloro	acylamino
	dimethylamino	chloro	dimethylamino
	dimethylamino	hydrogen	hydrogen
	hydrogen	hydrogen	dimethylamino
	trimethylammonium	hydrogen	hydrogen

and

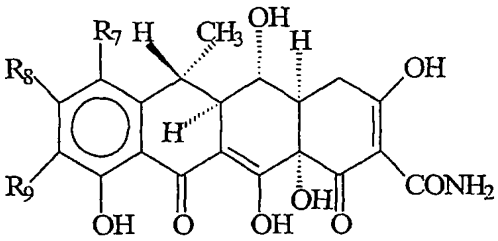
General Formula (II)



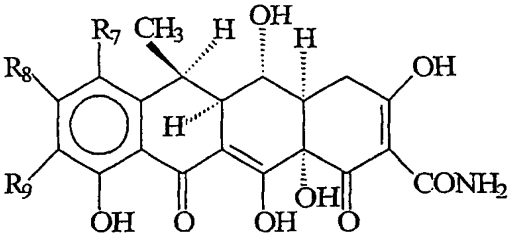
Structure L



Structure M



Structure N



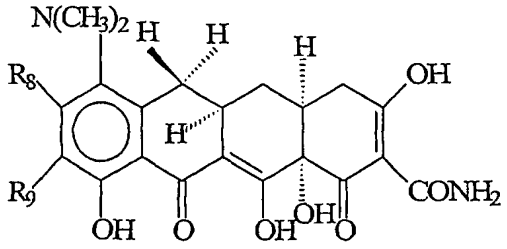
Structure O

wherein R7, R8, and R9 taken together in each case, have the following meanings:

R7	R8	R9
azido	hydrogen	hydrogen
dimethylamino	hydrogen	azido
hydrogen	hydrogen	amino
hydrogen	hydrogen	azido
hydrogen	hydrogen	nitro
dimethylamino	hydrogen	amino
acylamino	hydrogen	hydrogen
hydrogen	hydrogen	acylamino
amino	hydrogen	nitro
hydrogen	hydrogen	(N,N-dimethyl)glycylamino
amino	hydrogen	amino
hydrogen	hydrogen	ethoxythiocarbonylthio
dimethylamino	hydrogen	acylamino
hydrogen	hydrogen	diazonium
hydrogen	hydrogen	dimethylamino
diazonium	hydrogen	hydrogen
ethoxythiocarbonylthio	hydrogen	hydrogen
dimethylamino	chloro	amino
amino	chloro	amino
acylamino	chloro	acylamino
hydrogen	chloro	amino
amino	chloro	hydrogen
acylamino	chloro	hydrogen
monoalkyl amino	chloro	amino
nitro	chloro	amino

and

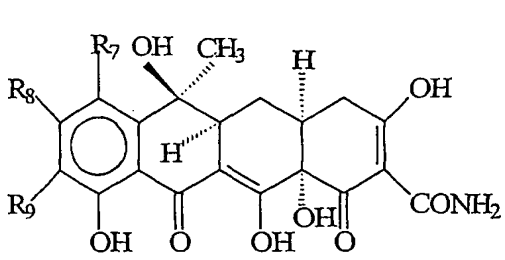
General Formula (III)



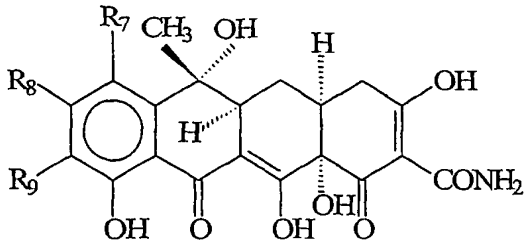
Structure P

wherein R8 is hydrogen or halogen and R9 is selected from the group consisting of nitro, (N,N-dimethyl)glycylamino, and ethoxythiocarbonylthio; and

General Formula (IV)



Structure Q



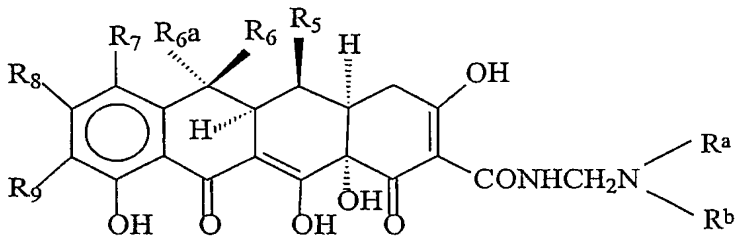
Structure R

wherein R7, R8, and R9 taken together in each case, have the following meanings:

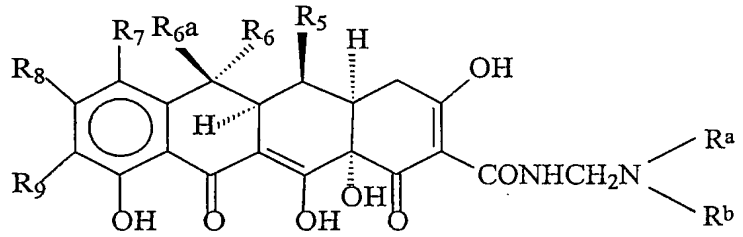
R7	R8	R9
amino	hydrogen	hydrogen
nitro	hydrogen	hydrogen
azido	hydrogen	hydrogen
dimethylamino	hydrogen	azido
hydrogen	hydrogen	amino
hydrogen	hydrogen	azido
hydrogen	hydrogen	nitro
bromo	hydrogen	hydrogen
dimethylamino	hydrogen	amino
acylamino	hydrogen	hydrogen
hydrogen	hydrogen	acylamino
amino	hydrogen	nitro
hydrogen	hydrogen	(N,N-dimethyl)glycylamino
amino	hydrogen	amino
diethylamino	hydrogen	hydrogen
hydrogen	hydrogen	ethoxythiocarbonylthio
dimethylamino	hydrogen	methylamino
dimethylamino	hydrogen	acylamino
dimethylamino	chloro	amino
amino	chloro	amino
acylamino	chloro	acylamino
hydrogen	chloro	amino
amino	chloro	hydrogen
acylamino	chloro	hydrogen
monoalkylamino	chloro	amino

nitro chloro amino

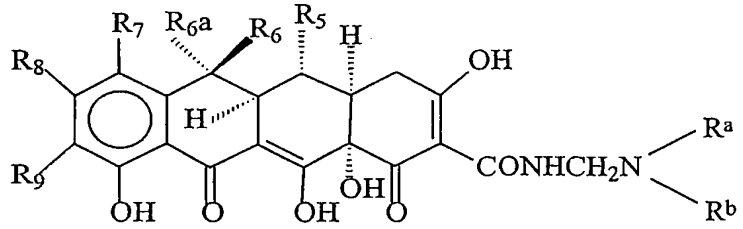
and pharmaceutically acceptable and unacceptable salts thereof.



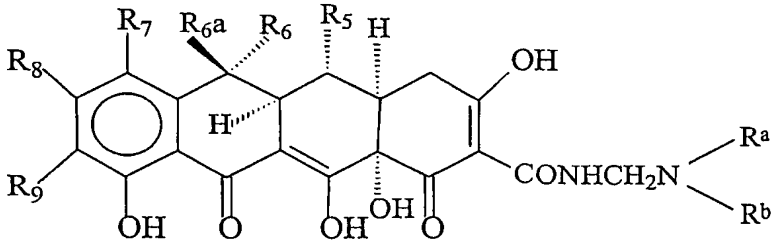
Structure S



Structure T



Structure U



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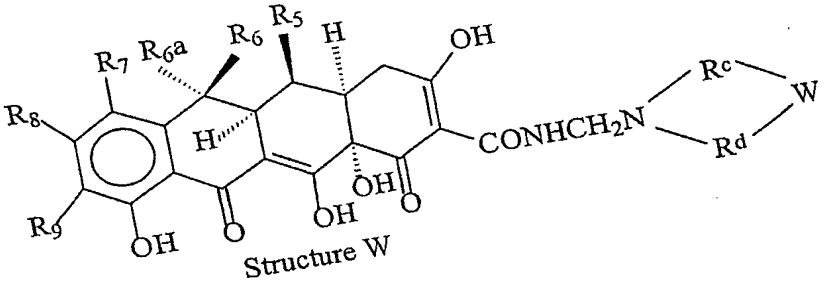
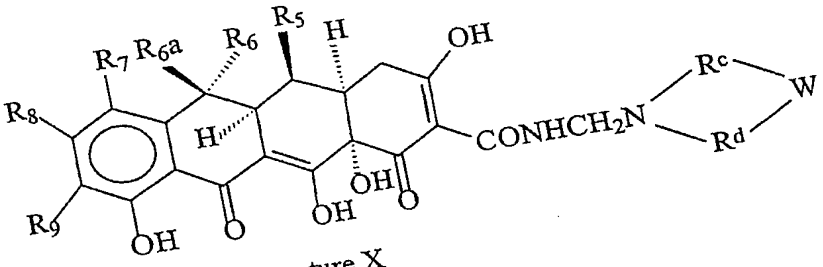
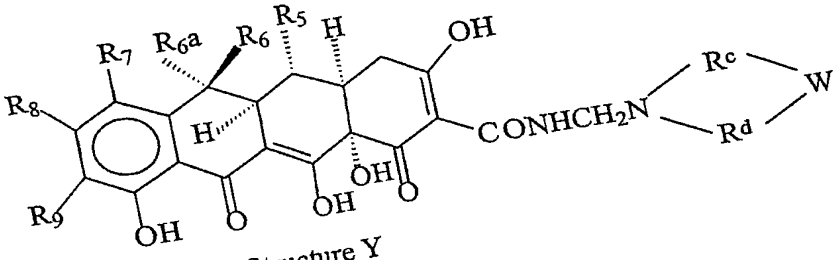


Structure W

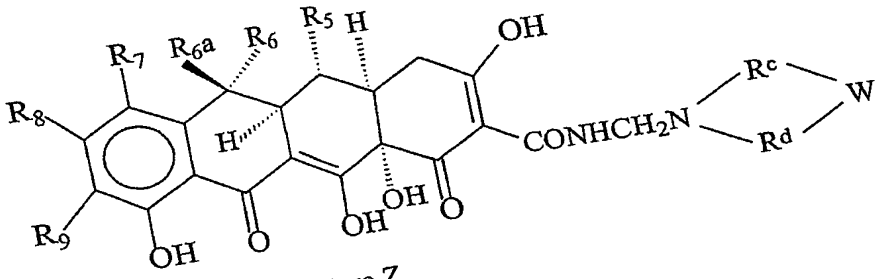


Structure X

5



Structure Y



Structure Z

wherein R7 is selected from the group consisting of hydrogen, amino, nitro, mono(lower alkyl) amino, halogen, di(lower alkyl)amino, ethoxythiocarbonylthio, azido, acylamino, diazonium, cyano, and hydroxyl; R6-a is selected from the group consisting of hydrogen and methyl; R6 and R5 are selected from the group consisting of hydrogen and hydroxyl; R8 is selected from the group consisting of hydrogen and halogen; R9 is selected from the group consisting of hydrogen, amino, azido, nitro, acylamino, hydroxy, ethoxythiocarbonylthio, mono(lower alkyl) amino, halogen, diazonium, di(lower alkyl)amino and $RCH(NH_2)CO$; R is hydrogen or lower alkyl; R^a and R^b are selected from the group consisting of hydrogen, methyl, ethyl, n-propyl and 1-methylethyl with the proviso that R^a and R^b cannot both be hydrogen; R^c and R^d are, independently $(CH_2)_nCHR^e$ wherein n is 0 or 1 and R^e is selected from the group consisting of hydrogen, alkyl, hydroxy, lower(C_1-C_3) alkoxy, amino, or nitro; and, W is selected from the group consisting of $(CHR^e)_m$ wherein m is 0-3 and R^c is as above, NH, N(C_1-C_3) straight chained or branched alkyl, O, S and N(C_1-C_4) straight chain or branched alkoxy; and pharmaceutically acceptable and unacceptable salts thereof. In a further embodiment, the following provisos apply: when either R7 and R9 are hydrogen then R8 must be halogen; and when R6-a, R6, R5 and R9 are all hydrogen and R7 is hydrogen, amino, nitro, halogen, dimethylamino or diethylamino, then R8 must be halogen; and when R6-a is methyl, R6 and R9 are both hydrogen, R5 is hydroxyl, and R7 is hydrogen, amino, nitro, halogen or diethylamino, then R8 is halogen; and when R6-a is methyl, R6 is hydroxyl, R5, R7 and R9 are all hydrogen, then R8 must be halogen; and when R6-a, R6 and R5 are all hydrogen, R9 is methylamino and R7 is dimethylamino, then R8 must be halogen; and when R6-a is methyl, R6 is hydrogen, R5 is hydroxyl, R9 is methylamino and R7 is dimethylamino, then R8 must be halogen; and when R6-a is methyl, R6, R5 and R9 are all hydrogen and R7 is cyano, then R8 must be halogen.

STRUCTURE K

wherein: R7, R8, and R9 taken together in each case, have the following meanings:

R7	R8	R9
hydrogen	hydrogen	amino
hydrogen	hydrogen	palmitamide

and

STRUCTURE L **STRUCTURE M** **STRUCTURE N** **STRUCTURE O**

wherein: R7, R8, and R9 taken together in each case, have the following meanings:

R7	R8	R9
hydrogen	hydrogen	acetamido
hydrogen	hydrogen	dimethylaminoacetamido
hydrogen	hydrogen	nitro
hydrogen	hydrogen	amino

and

STRUCTURE P

wherein: R8, and R9 taken together are, respectively, hydrogen and nitro.

STRUCTURE K:

wherein: R7, R8, and R9 taken together are, respectively, hydrogen, hydrogen and dimethylamino.

STRUCTURE C **STRUCTURE D** **STRUCTURE E** **STRUCTURE F**

wherein R7 is selected from the group consisting of an aryl, alkenyl and alkynyl; R6-a is selected from the group consisting of hydrogen and methyl; R6 and R5 are selected from the group consisting of hydrogen and hydroxyl; R8 is selected from the group consisting of hydrogen and halogen; R9 is selected from the group consisting of hydrogen, amino, azido, nitro, acylamino, hydroxy, ethoxythiocarbonylthio,

mono(lower alkyl) amino, halogen, diazonium, di(lower alkyl)amino and $RCH(NH_2)CO$; and pharmaceutically acceptable and unacceptable salts thereof;

or

STRUCTURE C **STRUCTURE D** **STRUCTURE E** **STRUCTURE F**

wherein: R7 is selected from the group consisting of hydrogen, amino, nitro, mono(lower alkyl) amino, halogen, di(lower alkyl)amino, ethoxythiocarbonylthio, azido, acylamino, diazonium, cyano, and hydroxyl; R6-a is selected from the group consisting of hydrogen and methyl; R6 and R5 are selected from the group consisting of hydrogen and hydroxyl; R8 is selected from the group consisting of hydrogen and halogen; R9 is selected from the group consisting of an aryl, alkenyl and alkynyl; and pharmaceutically acceptable and unacceptable salts thereof;

or

STRUCTURE C **STRUCTURE D** **STUCTURE E** **STRUCTURE F**

wherein: R7 and R9 are selected from the group consisting of an aryl, alkene, alkyne, or mixures thereof; R6-a is selected from the group consisting of hydrogen and methyl; R6 and R5 are selected from the group consisting of hydrogen and hydroxyl; R8 is selected from the group consisting of hydrogen and halogen; and pharmaceutically acceptable and unacceptable salts thereof.

STRUCTURE G **STRUCTURE H** **STRUCTURE I** **STRUCTURE J**

wherein R7 is selected from the group consisting of an aryl, alkenyl and alkynyl; R6-a is selected from the group consisting of hydrogen and methyl; R6 and R5 are selected from the group consisting of hydrogen and hydroxyl; R4 is selected from the group consisting of NOH, N-NH-A, and NH-A,where A is a lower alkyl group; R8 is selected from the group consisting of hydrogen and halogen;R9 is selected from the group consisting of hydrogen, amino, azido, nitro, acylamino, hydroxy,

ethoxythiocarbonylthio, mono(lower alkyl) amino, halogen, di(lower alkyl)amino and $RCH(NH_2)CO$; and pharmaceutically acceptable and unacceptable salts thereof;

or

STRUCTURE G **STRUCTURE H** **STRUCTURE I** **STRUCTURE J**

wherein R7 is selected from the group consisting of hydrogen, amino, nitro, mono(lower alkyl) amino, halogen, di(lower alkyl)amino, ethoxythiocarbonylthio, azido, acylamino, diazonium, cyano, and hydroxyl; R6-a is selected from the group consisting of hydrogen and methyl; R6 and R5 are selected from the group consisting of hydrogen and hydroxyl; R4 is selected from the group consisting of NOH, N-NH-A, and NH-A, where A is a lower alkyl group; R8 is selected from the group consisting of hydrogen and halogen; R9 is selected from the group consisting of an aryl, alkenyl and alkynyl; and pharmaceutically acceptable and unacceptable salts thereof;

or

STRUCTURE G **STRUCTURE H** **STRUCTURE I** **STRUCTURE J**

wherein: R7 and R9 are selected from the group consisting of an aryl, alkenyl, alkynyl; or mixtures thereof; R6-a is selected from the group consisting of hydrogen and methyl; R6 and R5 are selected from the group consisting of hydrogen and hydroxyl; R4 is selected from the group consisting of NOH, N-NH-A, and NH-A, where A is a lower alkyl group; and R8 is selected from the group consisting of hydrogen and halogen; and pharmaceutically acceptable and unacceptable salts thereof.

STRUCTURE K

wherein R7 is selected from the group consisting of an aryl, alkenyl and alkynyl; R8 is selected from the group consisting of hydrogen and halogen; R9 is selected from the group consisting of hydrogen, amino, azido, nitro, acylamino, hydroxy,

ethoxythiocarbonylthio, mono(lower alkyl) amino, halogen, di(lower alkyl)amino and $RCH(NH_2)CO$; and pharmaceutically acceptable and unacceptable salts thereof;

or

STRUCTURE K

wherein: R7 is selected from the group consisting of hydrogen, amino, nitro, mono(lower alkyl) amino, halogen, di(lower alkyl)amino, ethoxythiocarbonylthio, azido, acylamino, diazonium, cyano, and hydroxyl; R8 is selected from the group consisting of hydrogen and halogen; R9 is selected from the group consisting of an aryl, alkenyl and alkynyl; and pharmaceutically acceptable and unacceptable salts thereof;

or

STRUCTURE K

wherein: R7 and R9 are selected from the group consisting of an aryl, alkenyl, alkynyl and mixtures thereof; and R8 is selected from the group consisting of hydrogen and halogen; and pharmaceutically acceptable and unacceptable salts thereof;

and

STRUCTURE L **STRUCTURE M** **STRUCTURE N** **STRUCTURE O**

wherein: R7 is selected from the group consisting of an aryl, alkenyl and alkynyl; R8 is selected from the group consisting of hydrogen and halogen; and pharmaceutically acceptable and unacceptable salts thereof;

or

STRUCTURE L **STRUCTURE M** **STRUCTURE N** **STRUCTURE O**

wherein R7 is selected from the group consisting of hydrogen, amino, nitro, mono(lower alkyl) amino, halogen, di(lower alkyl)amino, ethoxythiocarbonylthio, azido, acylamino, diazonium, cyano, and hydroxyl; R8 is selected from the group consisting of hydrogen and halogen; R9 is selected from the group consisting of an aryl, alkenyl and alkynyl; and pharmaceutically acceptable and unacceptable salts thereof;

or

STRUCTURE L **STRUCTURE M** **STRUCTURE N** **STRUCTURE O**

wherein R7 is and R9 are selected from the group consisting of an aryl, alkenyl, alkynyl and mixtures thereof; R8 is selected from the group consisting of hydrogen and halogen; R9 is selected from the group consisting of hydrogen, amino, azido, nitro, acylamino, hydroxy, ethoxythiocarbonylthio, mono(lower alkyl) amino, halogen, di(lower alkyl)amino and $RCH(NH_2)CO$; and pharmaceutically acceptable and unacceptable salts thereof;

and

STRUCTURE P

wherein R9 is selected from the group consisting of an aryl, alkenyl and alkynyl; and R8 is selected from the group consisting of hydrogen and halogen; and pharmaceutically acceptable and unacceptable salts thereof;

and

STRUCTURE Q **STRUCTURE R**

wherein R7 is selected from the group consisting of an aryl, alkenyl and alkynyl; R8 is selected from the group consisting of hydrogen and halogen; R9 is selected from the group consisting of hydrogen, amino, azido, nitro, acylamino, hydroxy, ethoxythiocarbonylthio, mono(lower alkyl) amino, halogen, di(lower alkyl)amino and $RCH(NH_2)CO$; and pharmaceutically acceptable and unacceptable salts thereof;

or

STRUCTURE Q STRUCTURE R

wherein R7 is selected from the group consisting of hydrogen, amino, nitro, mono(lower alkyl) amino, halogen, di(lower alkyl)amino, ethoxythiocarbonylthio, azido, acylamino, diazonium, cyano, and hydroxyl; R8 is selected from the group consisting of hydrogen and halogen; R9 is selected from the group consisting of an aryl, alkenyl and alkynyl; and pharmaceutically acceptable and unacceptable salts thereof;

or

STRUCTURE Q STRUCTURE R

wherein R7 and R9 are selected from the group consisting of an aryl, alkenyl, alkynyl; and mixtures thereof; R8 is selected from the group consisting of hydrogen and halogen; and pharmaceutically acceptable and unacceptable salts thereof.

STRUCTURES S-Z

wherein R7 is selected from the group consisting of an aryl, alkenyl and alkynyl; R6-a is selected from the group consisting of hydrogen and methyl; R6 and R5 are selected from the group consisting of hydrogen and hydroxyl; R8 is selected from the group consisting of hydrogen and halogen; R9 is selected from the group consisting of hydrogen, amino, azido, nitro, acylamino, hydroxy, ethoxythiocarbonylthio,

mono(lower alkyl) amino, halogen, diazonium, di(lower alkyl)amino and $RCH(NH_2)CO$; R^a and R^b are selected from the group consisting of hydrogen, methyl, ethyl, n-propyl and 1-methylethyl with the proviso that R^a and R^b cannot both be hydrogen; R^c and R^d are, independently, $(CH_2)_nCHR^e$ wherein n is 0 or 1 and R^e is selected from the group consisting of hydrogen, alkyl, hydroxy, lower(C_1-C_3) alkoxy, amino, or nitro; and,W is selected from the group consisting of $(CHR^e)_m$ wherein m is 0-3 and said R^e is as above, NH, N(C_1-C_3) straight chained or branched alkyl, O, S and N(C_1-C_4) straight chain or branched alkoxy; and pharmaceutically acceptable and unacceptable salts thereof;

or

STRUCTURES S-Z

wherein R7 is selected from the group consisting of hydrogen, amino, nitro, mono(lower alkyl) amino, halogen, di(lower alkyl)amino, ethoxythiocarbonylthio, azido, acylamino, diazonium, cyano, and hydroxyl; R6-a is selected from the group consisting of hydrogen and methyl; R6 and R5 are selected from the group consisting of hydrogen and hydroxyl; R8 is selected from the group consisting of hydrogen and halogen; R9 is selected from the group consisting of an aryl, alkenyl and alkynyl; R^a and R^b are selected from the group consisting of hydrogen, methyl, ethyl, n-propyl and 1-methylethyl with the proviso that R^a and R^b cannot both be hydrogen; R^c and R^d are, independently, $(CH_2)_nCHR^e$ wherein n is 0 or 1 and R^e is selected from the group consisting of hydrogen, alkyl, hydroxy, lower(C_1-C_3) alkoxy, amino, or nitro; and, W is selected from the group consisting of $(CHR^e)_m$ wherein m is 0-3 and said R^e is as above, NH, N(C_1-C_3) straight chained or branched alkyl, O, S and N(C_1-C_4) straight chain or branched alkoxy; and pharmaceutically acceptable and unacceptable salts thereof;

or

wherein: R7 and R9 are selected from the group consisting of an aryl, alkenyl, alkynyl and mixtures thereof; R6-a is selected from the group consisting of hydrogen and methyl; R6 and R5 are selected from the group consisting of hydrogen and hydroxyl; R8 is selected from the group consisting of hydrogen and halogen; R^a and R^b are selected from the group consisting of hydrogen, methyl, ethyl, n-propyl and 1-methylethyl with the proviso that R^a and R^b cannot both be hydrogen; R^c and R^d are, independently, $(CH_2)_nCHR^e$ wherein n is 0 or 1 and R^e is selected from the group consisting of hydrogen, alkyl, hydroxy, lower(C_1-C_3) alkoxy, amino, or nitro; and W is selected from the group consisting of $(CHR^e)_m$ wherein m is 0-3 and said R^e is as above, NH, N(C_1-C_3) straight chained or branched alkyl, O, S and N(C_1-C_4) straight chain or branched alkoxy; and pharmaceutically acceptable and unacceptable salts thereof.

Throughout this specification, the descriptions of some structures include the term "lower alkyl." The term "lower alkyl" means an alkyl group comprising relatively few carbon atoms, for example, about one to ten carbon atoms. A preferred low end of this range is one, two, three, four or five carbon atoms; and a preferred high end of this range is six, seven, eight, nine or ten carbon atoms. Some examples of "lower alkyl" groups include methyl groups, ethyl groups, propyl groups, isopropyl groups, butyl groups, etc.

WHAT IS CLAIMED IS:

1. A method for treating an allergic reaction other than asthma in a mammal in need thereof comprising administering to said mammal a tetracycline compound in an amount that is effective to treat said allergic reaction.

2. The method according to Claim 1 wherein said allergic reaction results from inhaling an allergen.

3. A method according to Claim 1 wherein said mammal is a human.

4. A method according to Claim 1 wherein said treatment comprises administering said tetracycline compound systemically.

5. A method according to Claim 4, wherein said systemic administration is oral administration, intravenous injection, intramuscular injection, subcutaneous administration, transdermal administration or intranasal administration.

6. A method according to Claim 1, wherein said tetracycline compound is an antibiotic tetracycline compound administered in an amount which is 10-80% of the antibiotic amount.

7. A method according to Claim 6, wherein said antibiotic tetracycline compound is doxycycline, minocycline, tetracycline, oxytetracycline, chlortetracycline, demeclocycline, lymecycline or pharmaceutically acceptable salts thereof.

8. A method according to Claim 7, wherein said antibiotic tetracycline compound is doxycycline.

9. A method according to Claim 8, wherein said doxycycline is administered twice a day in a dose of 20 mg.

10. A method according to Claim 8, wherein said doxycycline is administered by sustained release over a 24 hour period.

11. A method according to Claim 8, wherein said doxycycline is administered in an amount of 40 milligrams once a day.

12. A method according to Claim 7, wherein said tetracycline compound is minocycline.

13. A method according to Claim 7, wherein said tetracycline compound is tetracycline.

14. A method according to Claim 1, wherein said tetracycline compound is an antibiotic tetracycline compound administered in an amount which results in a serum concentration which is 10-80% of the minimum antibiotic serum concentration.

15. A method according to Claim 14, wherein said antibiotic tetracycline compound is doxycycline, minocycline, tetracycline, oxytetracycline, chlortetracycline, demeclocycline, lymecycline or pharmaceutically acceptable salts thereof.

16. A method according to Claim 15, wherein said doxycycline is administered in an amount which provides a serum concentration in the range of about 0.1 to about 0.8 μg/ml.

17. A method according to Claim 15, wherein said doxycycline is administered in an amount which results in a serum concentration which is about 1 μg/ml.

18. A method according to Claim 15, wherein said minocycline is administered in an amount which results in a serum concentration which is about 0.8 μg/ml.

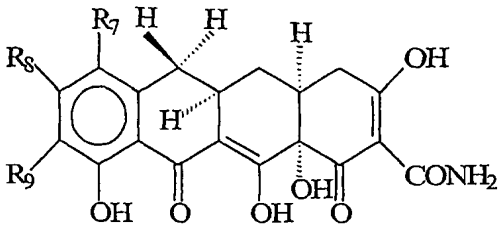
19. A method according to Claim 15, wherein said tetracycline is administered in an amount which results in a serum concentration which is about 0.5 μg/ml.

20. The method according to Claim 1, wherein said tetracycline compound has substantially no antibiotic activity.

21. A method according to Claim 20, wherein said non-antibiotic tetracycline compound is:

4-de(dimethylamino)tetracycline (CMT-1),
tetracyclinonitrile (CMT-2),
6-demethyl-6-deoxy-4-de(dimethylamino)tetracycline (CMT-3),
4-de(dimethylamino)-7-chlorotetracycline (CMT-4),
tetracycline pyrazole (CMT-5)
4-hydroxy-4-de(dimethylamino)tetracycline (CMT-6),
4-de(dimethylamino)-12α-deoxytetracycline (CMT-7),
6-α-deoxy-5-hydroxy-4-de(dimethylamino)tetracycline (CMT-8),
4-de(dimethylamino)-12α-deoxyanhydrotetracycline (CMT-9), or
4-de(dimethylamino)minocycline (CMT-10).

22. A method according to Claim 20 wherein said non-antibiotic tetracycline compound is selected from the group consisting of:

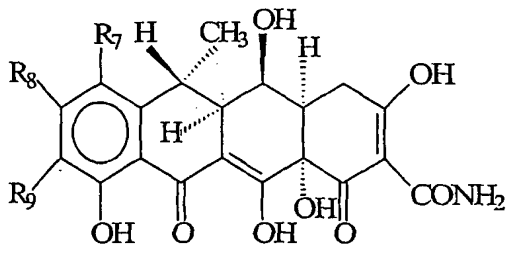


Structure K

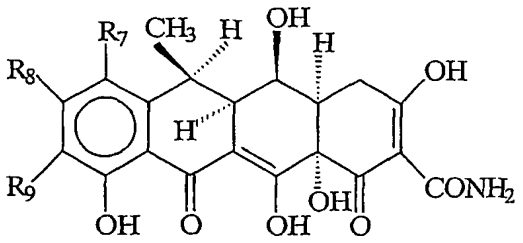
wherein: R7, R8, and R9 taken together in each case, have the following meanings:

R7	R8	R9
azido	hydrogen	hydrogen
dimethylamino	hydrogen	azido
hydrogen	hydrogen	amino
hydrogen	hydrogen	azido
hydrogen	hydrogen	nitro
dimethylamino	hydrogen	amino
acylamino	hydrogen	hydrogen
hydrogen	hydrogen	acylamino
amino	hydrogen	nitro
hydrogen	hydrogen	(N,N-dimethyl)glycylamino
amino	hydrogen	amino
hydrogen	hydrogen	ethoxythiocarbonylthio
dimethylamino	hydrogen	acylamino
dimethylamino	hydrogen	diazonium
dimethylamino	chloro	amino
hydrogen	chloro	amino
amino	chloro	amino
acylamino	chloro	acylamino
amino	chloro	hydrogen
acylamino	chloro	hydrogen
monoalkylamino	chloro	amino
nitro	chloro	amino
dimethylamino	chloro	acylamino
dimethylamino	chloro	dimethylamino
hydrogen	hydrogen	dimethylamino
dimethylamino	hydrogen	hydrogen
trimethylammonium	hydrogen	hydrogen

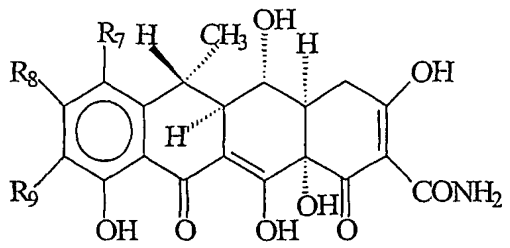
and



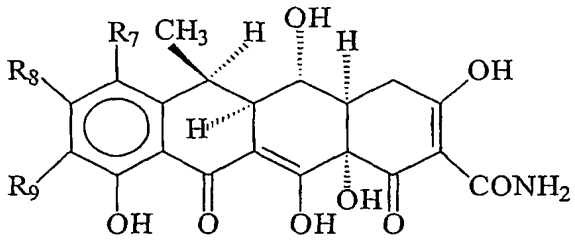
Structure L



Structure M



Structure N



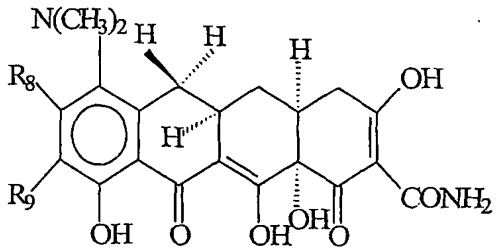
Structure O

wherein: R7, R8, and R9 taken together in each case, have the following meanings:

R7	R8	R9
azido	hydrogen	hydrogen
dimethylamino	hydrogen	azido
hydrogen	hydrogen	amino
hydrogen	hydrogen	azido
hydrogen	hydrogen	nitro
dimethylamino	hydrogen	amino
acylamino	hydrogen	hydrogen
hydrogen	hydrogen	acylamino
amino	hydrogen	nitro
hydrogen	hydrogen	(N,N-dimethyl)glycylamino
amino	hydrogen	amino
hydrogen	hydrogen	ethoxythiocarbonylthio
dimethylamino	hydrogen	acylamino
hydrogen	hydrogen	diazonium
hydrogen	hydrogen	dimethylamino
diazonium	hydrogen	hydrogen
ethoxythiocarbonylthio	hydrogen	hydrogen
dimethylamino	chloro	amino
amino	chloro	amino
acylamino	chloro	acylamino
hydrogen	chloro	amino

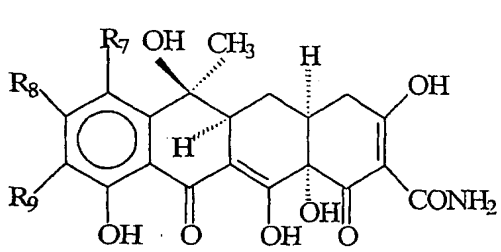
amino	chloro	hydrogen
acylamino	chloro	hydrogen
monoalkylamino	chloro	amino
nitro	chloro	amino

and

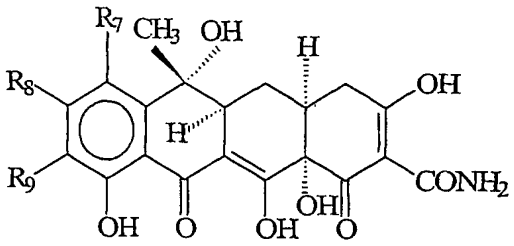


Structure P

wherein: R8 is hydrogen or halogen and R9 is selected from the group consisting of nitro, (N,N-dimethyl)glycylamino, and ethoxythiocarbonylthio; and



Structure Q



Structure R

wherein: R7, R8, and R9 taken together in each case, have the following meanings:

R7	R8	R9
amino	hydrogen	hydrogen
nitro	hydrogen	hydrogen
azido	hydrogen	hydrogen
dimethylamino	hydrogen	azido
hydrogen	hydrogen	amino
hydrogen	hydrogen	azido
hydrogen	hydrogen	nitro
bromo	hydrogen	hydrogen
dimethylamino	hydrogen	amino

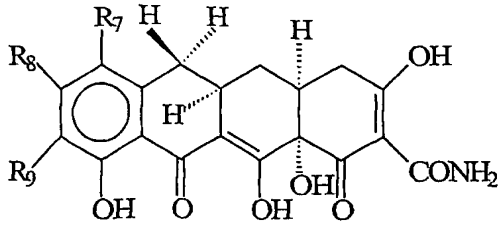
acylamino	hydrogen	hydrogen
hydrogen	hydrogen	acylamino
amino	hydrogen	nitro
hydrogen	hydrogen	(N,N-dimethyl)glycylamino
amino	hydrogen	amino
diethylamino	hydrogen	hydrogen
hydrogen	hydrogen	ethoxythiocarbonylthio
dimethylamino	hydrogen	methylamino
dimethylamino	hydrogen	acylamino
dimethylamino	chloro	amino
amino	chloro	amino
acylamino	chloro	acylamino
hydrogen	chloro	amino
amino	chloro	hydrogen
acylamino	chloro	hydrogen
monoalkylamino	chloro	amino
nitro	chloro	amino

and pharmaceutically acceptable salts thereof.

23. A method according to Claim 1, wherein said tetracycline compound has a photoirritancy factor of less than the photoirritancy factor of doxycycline.

24. A method according to Claim 1, wherein said tetracycline compound has a photoirritancy factor from about one to about two.

25. A method according to Claim 24, wherein said tetracycline compound has a general formula:

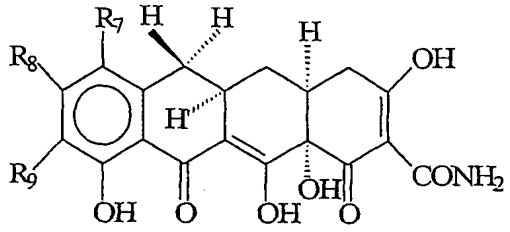


Structure K

wherein R7, R8, and R9 taken together are, respectively, hydrogen, hydrogen and dimethylamino.

26. A method according to Claim 1, wherein said tetracycline compound has a photoirritancy factor from about 1.0 to about 1.2.

27. A method according to Claim 26, wherein said tetracycline compound is selected from the group consisting of:

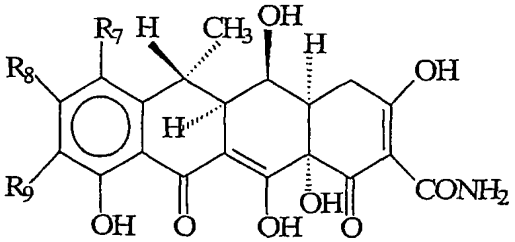


Structure K

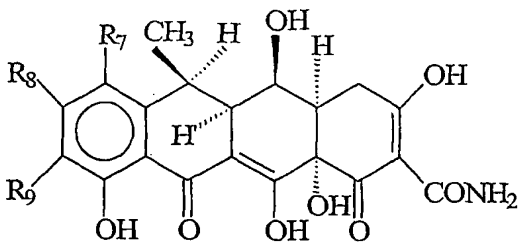
wherein R7, R8, and R9 taken together in each case, have the following meanings:

R7	R8	R9
hydrogen	hydrogen	amino
hydrogen	hydrogen	palmitamide

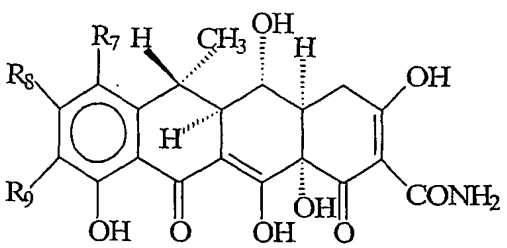
and



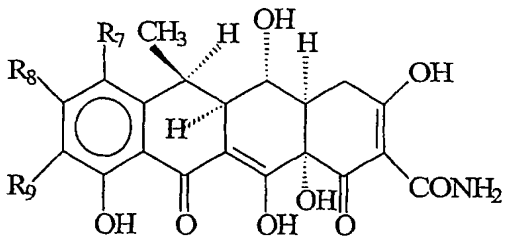
Structure L



Structure M



Structure N

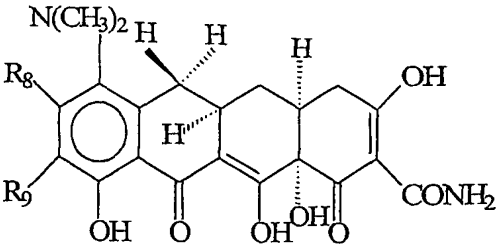


Structure O

wherein R7, R8, and R9 taken together in each case, have the following meanings:

R7	R8	R9
hydrogen	hydrogen	acetamido
hydrogen	hydrogen	dimethylaminoacetamido
hydrogen	hydrogen	nitro
hydrogen	hydrogen	amino

and



Structure P

wherein R8, and R9 taken together are, respectively, hydrogen and nitro.

28. A method according to Claim 1 wherein said treatment comprises administering said tetracycline compound topically.

29. A method according to Claim 28 wherein said tetracycline compound is administered in a mouthwash.

30. A method according to Claim 28 wherein said tetracycline compound is administered in an ocular solution.

31. A method of treating asthma in a mammal in need thereof comprising administering to said mammal a tetracycline compound in an amount that is effective to treat said asthma, without administering a bisphosphonate compound.

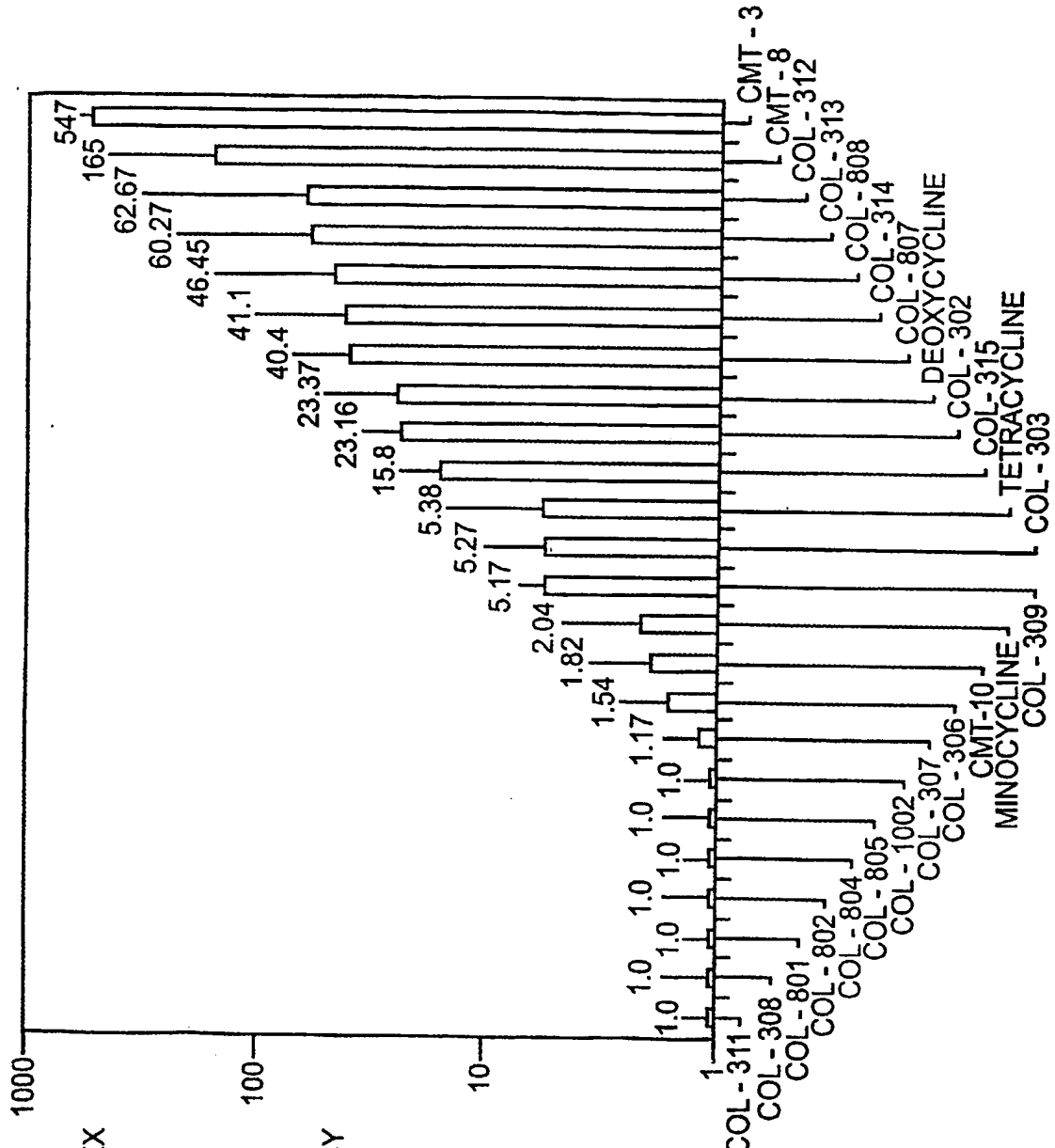


FIG. 1

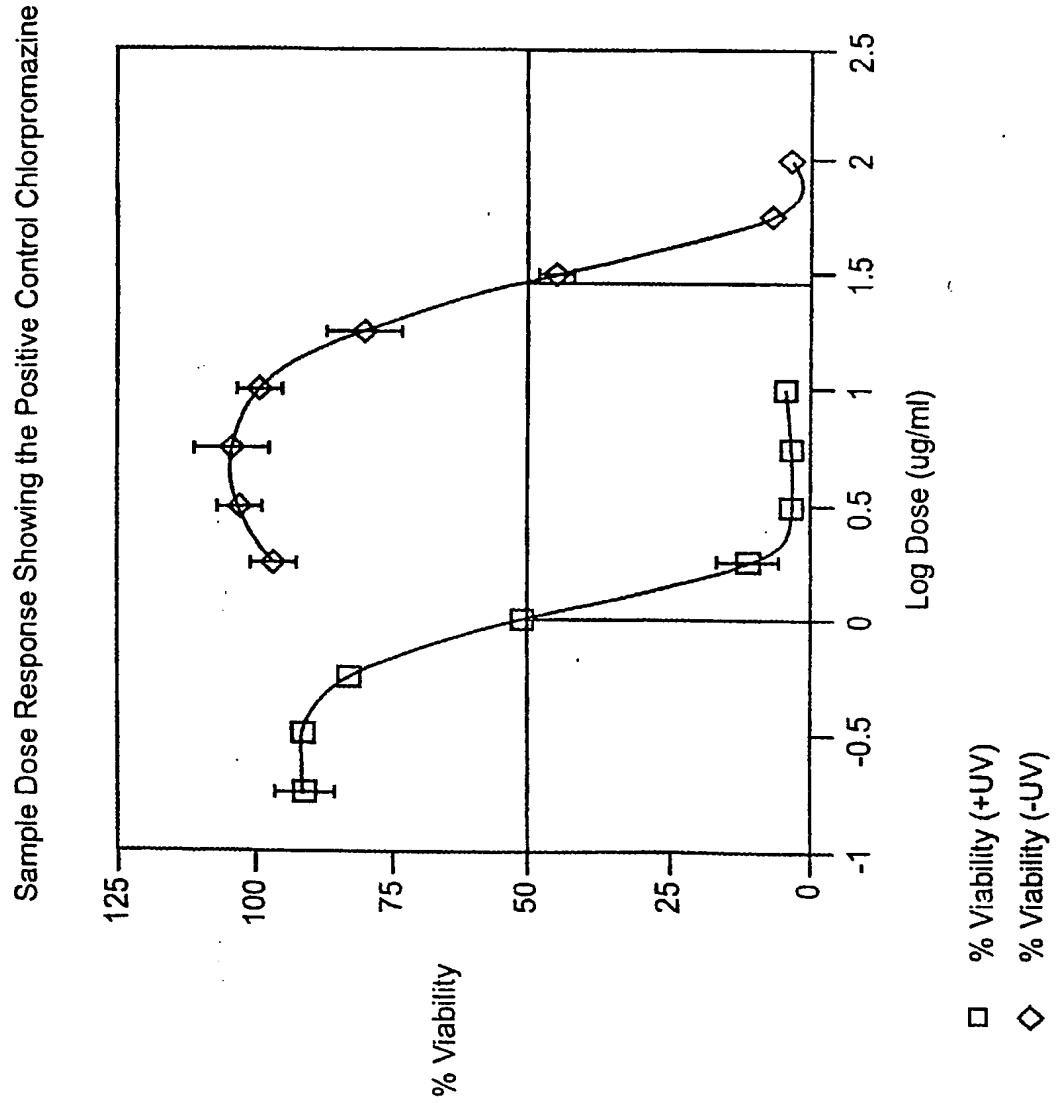


FIG. 2

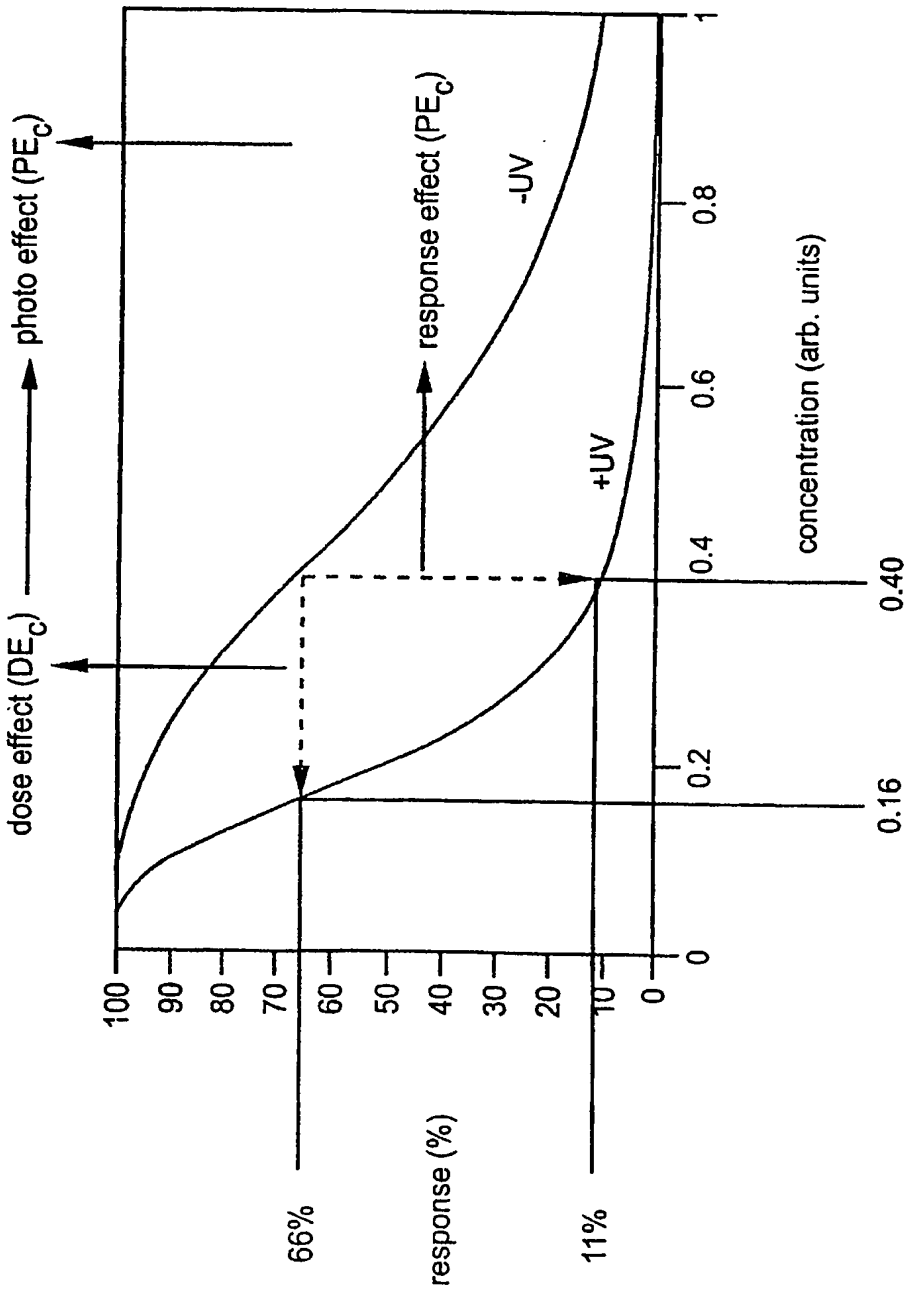


FIG. 3

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US03/15744

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) : A61K 31/195
US CL. : 514/563
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 514/563

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
WEST

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Database USPT, Accession Number 5,990,148 A, ISAKSON et al., Treatment of inflammation and inflammation-related disorders with a combination of a cyclooxygenase-2 inhibitor and a leukotriene A. sub.4 hydrolase inhibitor. 23 November 1999 (23.11.1999), see the entire document.	1-31

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
25 July 2003 (25.07.2003)	09 OCT 2003
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703)305-3230	Authorized officer Marianne Seidel Telephone No. (703) 308-1235

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(54) Title: METHODS OF TREATING ALLERGIC REACTIONS

(57) Abstract: A method for treating an allergic reaction other than asthma in a mammal need thereof comprising administering to said mammal a tetracycline compound in an amount that is effective to treat said allergic reaction.

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